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# MUTATIONS IN AGRICULTURAL PLANTS

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## INTRODUCTION.

THE pioneer work of STADLER on induced mutations is fundamental also to plant-breeding. Yet STADLER takes up an almost negative position in regard to their practical value. »But rare favourable mutation is likely to be accompanied by unfavourable mutations induced by the same treatment (1930, p. 18) . . . The result is a plant heterozygous for several genes, mostly undesirable.» (Even so late as 1943 a similar conception, though not so pronounced a one, was advanced by BURGEFF.) STADLER continues: »There is little chance of producing experimentally variations which have not already occurred in nature». He is however more optimistic as regards the breeding of fruit trees. These are vegetatively propagated; by means of induced mutations a new variation is directly created and gamete formation and fertilization are avoided.

American workers do not seem to have arrived at any positive results or conclusions, at least so far as agricultural plants are concerned (LINDSTROM, 1933; MAC ARTHUR, 1934; GENTER and BROWN, 1941). GOODSPEED and co-workers (1929, 1934, etc.), who induced and analysed numerous chromosome abnormalities in *Nicotiana*, have not continued their investigations in this field.

Several Russian investigators have been considerably more positive in their attitude, first and foremost DELAUNAY and SAPEHIN, who in a series of papers described vital mutants in wheat, several of which possess practical interest. The former published in 1934 a synoptical work which, according to a review in *Resumptio Genetica*, 10, p. 164, stresses the value of induced mutants for plant-breeding. Yield tests do not seem to have been carried out. In any case no crop figures have been published.

German research-workers began their experiments with induced mutations at an early time. To begin with, however, their works were more of a theoretical character. First in the years 1942 and 1943 FREISLEBEN and LEIN described extensive model experiments in barley. After X-ray radiation and mass infection they even succeeded in pro-

ducing a mildew-resistant line. Much earlier, SENGBUSCH and HACKBARTH had shown that spontaneous mutants can successfully change a species and thereby improve its agricultural value (in their case sweet lupine).

The German works on induced vital mutants were first undertaken on a large scale after a lecture held by NILSSON-EHLE in the year 1939 in Halle on polymeric factors in barley. Unfortunately, this lecture was only published in the form of a short notice. As early as the mid-thirties he produced vital mutants (p. 16), at least one of which was found to be equal to the mother-line in yield. Crosses with the very straw-stiff malt-barley line Kenia have given, according to a personal communication, products with still higher straw-strength and, simultaneously, very good productivity.

The experiments which underlie the present work, and which have further shown the practical value of the induced mutants, were commenced early (1929—30) and have been promoted in various ways by NILSSON-EHLE. Since January, 1940, the cost of all the work on induced mutations has been defrayed from funds donated by A.B. Salt-sjöqvarn, Stockholm, and A. B. Mårten Pehrsons Valsqvarn, Kristianstad, through the medium of the Head of the Swedish Seed Association, Professor Å. ÅKERMAN. This has rendered it possible to extend the experiments considerably. The practical work is being conducted in the different departments of the Swedish Seed Association. Mutants in barley, wheat, oats and flax appear to be especially promising from a practical point of view. But also in soybeans, sweet lupine and oleiferous species of the Cruciferae mutants of interest have been observed. Here as elsewhere results are obtained first after lengthy and extensive experiments.

Certain analyses for this work have been carried out by Statens Centrala Frökontrollanstalt (the State Seed Testing Station) (Professor G. NILSSON-LEISSNER) as well as by A.B. Stockholms Bryggerier (H. THUNÆUS, Engineer). The heads of the branch stations of the Seed Association have carried out special yield trials.

Of recent years the irradiation experiments have been conducted by the Radiophysics Institute, Stockholm (Director: Professor R. SIEVERT), under the supervision of Dr. A. FORSSBERG.

In the account of the work submitted here theoretical and practical results have been interwoven. In spite of its size this paper gives merely a *survey* of the results rather than an *exhaustive account*. The physical

and mathematic problems, related to X-ray irradiation, are discussed in the work of LEA (1946), to which the reader is referred.

## I. EFFECT OF X-RAY RADIATION IN THE $X_1$ GENERATION.

According to the definition applied in this paper plants raised from X-rayed seeds form an  $X_1$  generation. The  $X_1$  plants are often heterozygous for induced genome changes of various kinds. After self-fertilization they give rise to an  $X_2$  generation. In this or in later generations ( $X_3$ ,  $X_4$ ) mutants segregate out. Mutations can also be induced by irradiation of pollen-grains and egg-cells. This method is more circumstantial and is not particularly suitable for practical purposes.

### 1. COURSE OF MITOSIS IN IRRADIATED SEEDS.

X-rayed dry seeds germinate even when they have received very high X-ray doses. In *Avena*, for instance, the radicle and the rootlets take up water, pierce the coleorhiza and after 75.000 r still show nuclear divisions, although these run an extremely irregular course. However much the interphase chromosomes of the resting seeds may have been affected or fragmented, the cells can nevertheless begin their prophase complete metaphase and anaphase and enter telophase and interphase stages. After that, however, the most damaged cell-nuclei degenerate or become inactivated, so that after a few mitotic cycles the division have an almost normal appearance. Interphase chromosomes may thus be broken into pieces without this influencing the immediate capacity for division to any notable extent.

Irradiation of resting seeds causes solely the so-called secondary effect, i. e. the chromosomes are fragmented and translocated (or inverted) in the numerous breaks that have arisen. Irradiation of nuclear divisions while in progress, on the other hand, is followed by the *primary effect* with its irregular fragmentation and agglutination of chromosomes («stickiness»), the *mitose-free intermediate period* when division ceases, and the *secondary effect*, which is observed in those cells which were in interphase stage on being rayed. The longer the irradiation has been, the longer will the mitose-free period be. As dry seeds contain only resting nuclei, it is evident that they can show nothing but the secondary effect. From works recently published by EULER, HEVESY and their associates (1944, 1945) we know that the mitose-free period occurs because desoxyribose nucleotides are formed. A certain quantity of nucleotides must be present for mitoses to start.

GUSTAFSSON (1937), GELIN (1941) as well as FRÖIER, GELIN and GUSTAFSSON (1941) have reported results concerning the frequency

disturbances in irradiated seeds. GELIN observed in Golden barley (Gullkorn), treated with 10.000 r-units, 13 % and 28 % disturbed anaphases at the first division of the cells, the lower frequency in seeds having a water content of 10 %, the higher in seeds with a water content of 15 %. FRÖIER, GELIN and GUSTAFSSON found that an identical dosage was followed by fewer disturbances in diploid species than in tetraploids, and fewer in the latter than in hexaploids, and that the number of disturbances induced by low doses is proportional to the number of genomes (cf. also SMITH, 1943). A dosage of 5.000 r gave 11 % disturbed cells in *Triticum monococcum* ( $2n=14$ ), on an average 22 % in *T. durum* and *dicoccum* ( $2n=28$ ), and 29 % in *T. vulgare* ( $2n=42$ ). Reduced to the diploid level ( $2n=14$ ) the figures will be 11,0—11,1—9,8 %. The immediate frequency of disturbances is already rather high at this dose. At 25.000 r it amounted to 99 % in *T. durum*, 96 % in *T. vulgare* and 96 % in *Avena sativa*. Thus, after this X-ray dose practically all observed cell divisions were badly damaged.

If the seeds have been steeped in water before irradiation, or their water content is raised in some other way, the mitotic disturbances also increase in number. This also takes place if the seeds are drenched with metallic salts or pretreated with various cell-poisons. In a certain experimental series with Golden barley GELIN (1941) obtained disturbance frequencies of 13, 28, 54 and 51 % respectively in seeds containing 10 %  $H_2O$ , 15 %  $H_2O$ , or that had been steeped in water or heteroauxin for 24 hours. Pretreatment of this kind also raises the mutation frequency.

The extent to which the external environmental conditions can influence the disturbance effect has been clarified by GELIN in a series of practically important but as yet unpublished investigations. Among other things he has shown that chromosome disturbances increase substantially in number if the temperature falls towards zero at germination, but that they decrease if the temperature is raised. The same result has been obtained by SAX (1939) after irradiation of pollen-grains of *Tradescantia*.

## 2. SEEDLING LETHALITY, SPROUTING ABILITY AND $X_1$ STERILITY.

For all mutation work it is of importance to fix suitable irradiation doses. The X-ray effect can be determined either by means of laboratory tests of germination and sprouting ability, the dose being settled that reduces the sprouting ability of the  $X_1$  plants to a suitable degree, or by field experiments in which the number of plants harvested after differ-

ent X-ray doses is calculated. These two testing methods do not give the same result because field material is always exposed to considerably harder conditions. Only field tests give a fully reliable verdict. For preliminary investigations or for work of a special nature laboratory tests will suffice.

Different kinds of plants react very dissimilarly to X-radiation (GUSTAFSSON, 1944). Especially insensitive are the seeds of the Cruciferae, which in certain cases (white mustard and swedes) stand doses of wellnigh 100.000 r and even more. On the other hand, the seeds of the pea plants are very sensitive, their maximum dose being only 10.000—20.000 r. The same applies to the seeds of hemp, sunflowers and safflowers, which cannot endure 10.000 r. For practical work we have tried to fix what may be called the »critical dose», i. e. the dose that while still producing a sufficiently large field material induces a maximum of mutations and chromosome re-arrangements. The »critical dose» falls with increasing water content of the seeds.

Types of plants having seeds that can stand high X-ray irradiation are also rich in fatty acids (oil). Naturally this does not mean that the oil content alone determines the difference in susceptibility. Rather is this a phenomenon that depends partly on the family to which the plant belongs, partly on the general chemical properties of the seeds, including the oil content and water content.

For the common cereals wheat, oats, barley and rye an X-ray dose of 10.000—20.000 r is the most suitable. The effect varies however considerably in different years. For winter wheat and winter rye this is quite natural, as the injured seedlings have to bear the cold of winter. In spring barley, spring wheat and oats the result also varies with the nature of the soil and the climatic conditions during the period of vegetation.

A special method, particularly applicable to species with ray-resistant seeds, consists in having the irradiated seeds stored for one or more years before they are sown. This procedure has been tried with advantage in white mustard, swedes and rapè. The long storage has the result that the X-ray effect is substantially intensified.

In proportion as the X-raying increases in intensity various inhibitions of development make their appearance. These manifest themselves in such manner that the sprouting capacity falls, the first leaves become yellow and shrivelled, often the sprout does not pierce the surface of the soil, or, if this is the case, it dies at an early stage, etc. (The yellowing of the leaves does not solely depend upon the genomatic dis-

turbances but is also connected with the inactivation or destruction of the plastids, see e.g., HRUBY, 1935.) The plants that survive develop more or less sterile inflorescences and fruits depending on the quantity of irradiation, and are now and then abnormal in other respects (ramified, stunted, lacking chlorophyll). Photographs illustrating the X-ray effects can be found in FREISLEBEN and LEIN (1943) and GUSTAFSSON (1944).

The relation between the fertility of the  $X_1$  plants and the X-ray dose given has been determined in two extensive series of two-rowed

TABLE 1. *X-ray dosage and  $X_1$  fertility.*

	M a j a b a r l e y				Ymer barley, line 40/13 b <sub>7</sub>			
	Per cent harvested plants	Average fertility %	Per cent normally fertile plants	N	Per cent harvested plants	Average fertility %	Per cent normally fertile plants	N
Control.....	75	97, <sub>1</sub>	98	90	?	96, <sub>1</sub>	100	25
500 r.....	—	—	—	—	51	94, <sub>2</sub>	92	50
1.000 r.....	—	—	—	—	61	92, <sub>8</sub>	80	50
2.500 r.....	64	89, <sub>5</sub>	63	557	57	91, <sub>8</sub>	74	50
5.000 r.....	32	77, <sub>6</sub>	30	277	42	82, <sub>0</sub>	36	50
7.500 r.....	—	—	—	—	44	75, <sub>2</sub>	22	50
10.000 r.....	16	75, <sub>4</sub>	14	142	41	75, <sub>6</sub>	12	203
15.000 r.....	13	73, <sub>8</sub>	10	91	39	68, <sub>7</sub>	10	196
20.000 r.....	10	74, <sub>1</sub>	9	140	37	61, <sub>9</sub>	2	185
25.000 r.....	8	66, <sub>5</sub>	4	119	37	58, <sub>8</sub>	1	374

barley. One was Maja barley (GUSTAFSSON, 1942) with six doses from 2.500—25.000 r, the other was a sister strain to Ymer barley (40/13 b<sub>7</sub>) with nine doses from 500—25.000 r. In both these series the fertility was examined ear for ear and plant for plant by counting the number of ripe grains and the number of empty florets. Certainly this method is rather tedious, but it is the only fully reliable one. As Table 1 shows, when given low doses the two series agree so far as the average fertility and the number of fully fertile plants (90—100 %) are concerned. Under the highest doses Ymer b<sub>7</sub> has a lower average fertility and a lower number of fully fertile plants. On the other hand, the number of harvested plants is considerably higher in Ymer b<sub>7</sub> barley than in Maja. No doubt this partly stands in connexion with the different environmental conditions of 1940 and 1943, though different seed properties also exercise some influence.

FREISLEBEN and LEIN (l.c.) emphasize the great difference between their  $X_1$  results and those submitted by GUSTAFSSON (1942). However, the differences in fertility are not very conspicuous, especially if their figures are corrected for the overflow fertility in the control line (87 % as against actually 95—98 %). The number of harvested plants, however, is considerably lower in their experiments.

A point of methodological interest is that the number of spikelets (florets) changes with the X-ray dose. The sterility is annually determined in the  $X_1$  plants of two-row barley as exactly as is ever possible. These determinations give simultaneous data as to the number of ears per plant and the number of spikelets per ear. A survey is given below showing that the control material of the year 1940 contained on an average 23,98 spikelets per spike. Following a 2,500 r dose the number fell considerably (the difference amounts to 2,10 spikelets) in order later on to rise gradually to 27,32 and 27,07 spikelets per ear. The differences from the controls are statistically significant in the case of all doses except 5,000 r.

		Difference from controls	D/m
Control .....	23,98 $\pm$ 0,29 spikelets	—	—
2.500 r .....	21,88 $\pm$ 0,29 »	2,10 $\pm$ 0,41	5,12
5.000 r .....	23,73 $\pm$ 0,25 »	0,25 $\pm$ 0,38	0,66
10.000 r ....	24,93 $\pm$ 0,29 »	0,95 $\pm$ 0,41	2,32
15.000 r ....	26,17 $\pm$ 0,37 »	2,19 $\pm$ 0,47	4,66
20.000 r .....	27,32 $\pm$ 0,29 »	3,34 $\pm$ 0,37	9,03
25.000 r ....	27,07 $\pm$ 0,32 »	3,09 $\pm$ 0,43	7,19

(Maja barley X-rayed 1940; see also GUSTAFSSON, 1942 a, Table 1.)

The cause of these differences is no doubt simply as follows. FRÜIER and GUSTAFSSON (1944) have shown that the larger the X-rayed seeds are, the more will they endure irradiation; germinability and sprouting ability are better. In an ordinary sample of seeds that have not been sieved hard the smaller seeds will be the more damaged. After low doses, however, they are able to go on living, their  $X_1$  plants put forth ears and set seed but, owing to their general weakness, give fewer spikelets than normally. Under high doses the X-rayed sprouts are killed early, and only the  $X_1$  plants that have arisen from vigorous seeds remain alive. Their vitality enables them to develop abundant spikelets. Among other signs indicating that an enormous selection takes place is the fact that after treatment with 25.000 r only 8 % of all rayed seeds gave mature  $X_1$  plants. The corresponding figure for the control was 75 %. An ordinary unsieved lot of seeds will show con-

siderable differences in size. As a consequence a strong selection should set in according to the X-ray susceptibility of the seeds.

In their just cited experiments FREISLEBEN and LEIN have not evaluated the fertility in the  $X_1$  generation by sufficiently exact methods. Instead of the *direct sterility* ear for ear, they determined the average number of grains per plant. This is a faulty method which may lead to errors. If for some reason the  $X_1$  fertility is to be analysed in order to correlate it with the type or frequency of mutation, the more roundabout method is the only correct one.

### 3. SEED PROPERTIES AND THEIR INFLUENCE ON THE X-RAY SUSCEPTIBILITY.

*Water content.* — Since STADLER's investigations (1928 b) it has been known that the mutation frequency rises considerably if the seeds are soaked in water before being irradiated. This fact has since been demonstrated by several investigators, e. g. GUSTAFSSON (1940). The increased susceptibility has been clarified by WERTZ (1940) in a careful methodological investigation. KAPLAN (1940) has studied the X-ray injuries that follow water treatment and irradiation of *Antirrhinum* pollen by determining the different germinability of the grains. (See also KNAPP and KAPLAN, 1942.)

In mutation experiments the seeds that have been soaked in water before irradiation show an inferior sprouting ability, a higher number of dead seedlings and a higher  $X_1$  sterility than seeds irradiated when their water content is low. Two Svalöf experiments have made this clear. One was a comparison between series of Golden barley in which the seeds contained 10 % of  $H_2O$ , were soaked in distilled water for 24 hours, and treated with 0.01 % heteroauxin for the same period of time. The dose of irradiation amounted to 5.000 and 10.000 r (GUSTAFSSON, 1940). Series of seeds from Maja barley (1940) were also treated in the same manner. The number of harvested plants was decidedly higher in the dry-seed series than in those steeped in water, although the difference was most accentuated after 10.000 r. As regards the fertility, this showed a substantial fall in the water-treated series. Here, again, the differences were statistically significant. The auxin-treated and the water-treated series behaved similarly.

Small differences in water content also influence the intensity of the X-ray injuries. In the 1939 experiments with Golden barley seed samples were also rayed that contained 15 % of  $H_2O$  and that had been stored at this water content for some time. The number of harvested plants did not fall notably, whereas this was the case with the mean fertility. The difference in fertility between series B and A

TABLE 2. *The fertility of the X<sub>1</sub> generation after different pre-treatment of the seeds.*  
(Ymer b<sub>7</sub>).

Treatment	Fertility of $X_1$ plants											Average $X_1$ fertility	N
	0	10	20	30	40	50	60	70	80	90	100 %		
Control.....	—	—	—	—	—	—	—	—	—	—	100 %	96.4 ± 0.4 %	25
80° for 72 hours.....	—	—	—	—	—	—	—	—	—	8,0 %	92,0 %	94.5 ± 0.5 %	25
80° for 144 hours.....	—	—	—	—	—	—	—	—	—	4,0 %	96,0 %	94.3 ± 0.5 %	25
80° for 216 hours.....	—	—	—	—	—	—	—	—	—	—	100 %	95.0 ± 0.3 %	25
11 % $H_2O$ : 10.000 r .....	—	—	8,0 %	10,0 %	14,0 %	23,0 %	22,0 %	16,0 %	4,0 %	3,0 %	—	57,0 ± 1.5 %	100
$P_2O_5$ : 10.000 r .....	—	2,0 %	6,0 %	14,0 %	26,0 %	18,0 %	14,0 %	14,0 %	4,0 %	2,0 %	—	53,2 ± 2.4 %	50
80° 24 hours: 10.000 r.....	—	—	—	1.5 %	0,8 %	4,6 %	4,6 %	17,6 %	46,6 %	24.4 %	—	82,3 ± 1.1 %	131
80° 72 hours: 10.000 r.....	—	—	—	—	—	1,0 %	4,1 %	16,3 %	42,9 %	35,7 %	—	85,8 ± 0.9 %	98
80° 144 hours: 10.000 r.....	—	—	—	—	0,6 %	1,3 %	4,5 %	16,0 %	41,7 %	35,9 %	—	85.5 ± 0.8 %	156

(seed material with respectively 10 and 15 %  $H_2O$ ) amounted at 5.000 r to  $2,96 \pm 0,59$  % with  $D/m = 5,0$  and at 10.000 r to  $6,68 \pm 0,69$  % with  $D/m = 9,7$ .

A desiccation of the seeds before irradiation ought to have the opposite effect to that of soaking them in water and should accordingly reduce the susceptibility. No complete experimental series have as yet been carried out in which the water content has been gradually reduced. On the other hand, an investigation was made in the spring of 1943 into the effect of X-ray irradiation combined with an intense desiccation by heat (Table 2).

A series of seed samples of Ymer  $b_7$  was taken on March 17 for different pretreatments. On this occasion the water content was rather high (16,9 %). The control material was stored at room temperature, and immediately before the X-raying (March 26) the water content amounted to 11,3 %. On March 17 six boxes, each containing 300 grains in one layer, were placed in a thermostat, gradually given a temperature of 80° C. and kept there 1, 3, 6 and 9 days. Thereupon two control series and three of the heat-treated samples (1, 3 and 6 days) were given an X-ray dose of 10.000 r. The fertility was then determined in all  $X_1$  plants that had arisen in the series subjected to combined heat and irradiation. In those control series which had been given solely 10.000 r  $2 \times 50$  plants were examined for fertility, these plants being naturally taken out entirely at random.

The unrayed control series gave, as in other cases, a very high fertility (96 %). In the three series in which the grains had been merely heat-treated the fertility was somewhat reduced. Statistically, however, the differences were doubtless significant ( $D/m = 2,95$ ,  $3,06$  and  $2,88$ ). Solely heat treatment thus reduces the fertility of the plants that arise. This in its turn is probably due to the fact that the chromosomal re-arrangements in the heat-treated seeds are considerably increased, and it agrees with earlier results obtained at Svalöf and elsewhere (see, e. g., PETO, 1937) and can be explained in a similar way as the temperature coefficient for the mutation process (TIMOFÉEFF-RESSOVSKY, 1937).

Irradiation with solely 10.000 r lowered the fertility from 96 % to 57 %. The great decrease of the fertility in just this experiment is probably associated with the high water content the seeds had when the samples were taken. Both the series given 10.000 r behaved in the same way. The average fertility in the two cases was 54 % and 59 % and the plants were distributed similarly over different fertility ranges.

X-ray treatment combined with a previous heat treatment increased the fertility very considerably, from 57 % to 82 %, 86 % and 86 % in the three heat-treated series. This is the more remarkable as heat treatment alone *reduces* fertility. The increased fertility after heat

treatment and irradiation is probably due to a loss of water in the cells, nuclei and, maybe, the chromosomes. In its turn this change brings about increased chromosome stability and fewer disturbances at the subsequent germination.

The fertility especially increased after heat treatment for three days. After treatment for 24 hours the average fertility was lower than after that lasting three and six days. The D/m for the difference between 1 and 3 days amounts to 2,53, for the difference between 1 and 6 days to 2,40.

TABLE 3. *Seed age and X-ray effect (Maja barley).*

Treatment	Small seeds (Sieve 2,2 mm.)			Large seeds (Sieve 2,8 mm.)		
	Per cent harvested X <sub>1</sub> plants	No. of harvested X <sub>1</sub> plants	Average X <sub>1</sub> ear fertility %	Per cent harvested X <sub>1</sub> plants	No. of harvested X <sub>1</sub> plants	Average X <sub>1</sub> ear fertility %
Control 1943 .....	69	62	96,1	71	64	92,9
1942 .....	67	60	96,0	83	75	98,5
1941 .....	66	59	97,8	67	60	97,6
1940 .....	39	35	97,7	33	30	98,3
1939 .....	30	27	92,3	41	37	88,7
5.000 r 1943 .....	46	139	76,3	50	150	77,4
1942 .....	48	145	69,4	57	172	80,6
1941 .....	30	89	79,2	50	149	83,0
1940 .....	13	40	62,5	22	65	76,2
1939 .....	10	29	55,3	12	36	65,3

SMITH, 1943, has likewise shown that heat treatment *before* irradiation increases the resistance. On the other hand, this is reduced if the heat treatment comes *after* the irradiation.

*Age.* — In 1944 an experiment was laid down to test the X-ray susceptibility in aged seed. The initial material was Maja barley seeds in two sizes (sieves 2,8 and 2,2 mm.), derived from the 1943, 1942, 1941, 1940 and 1939 crops, the samples being stored in the same way and having identical water contents. The X-ray doses amounted to 5.000 (and 10.000) r. Each irradiated seed-series consisted of 300 seeds, each control-series of 90 seeds. The fertility was determined within each group only on one ear per plant.

From Table 3 it is seen that the germinability of the aged control-seeds falls sharper in the small-seeded than in the large-seeded series. In the four youngest series the ear fertility is not reduced but is about 95 %.

After a dosage of 5.000 r the differences are very expressive. In all cases the

small-seeded series are inferior, this condition being most pronounced in material of the years 1941 and 1940. The average ear fertility is about equally high in the four youngest year-classes of each group, but distinctly reduced in the 1939 series.

Rather irregular behaviour is shown by the series at 10,000 r. A characteristic feature, however, is that in both the parallel series the oldest material has not yielded a single harvested plant. In the other year-classes the small seeds are considerably more susceptible than the large ones. The mean ear fertility in the series containing a sufficient material lies at 50–60 %.

*Size of seed.* — On several occasions above stress has been laid on the significance of the size of the seed for X-ray susceptibility. In the cases hitherto examined (barley, see above, also unpubl.; wheat, see FRÖIER and GUSTAFSSON, 1944) it has been shown that small though well matured and uninjured seeds are more liable to lose their vitality after even moderate doses than medium-sized seeds and these, in their turn, more easily than large seeds. The cause is probably that even at comparatively high X-ray doses numerous uninjured cells remain in the largest embryos and, on account of their livelier activity of division, replace or push aside cells that have been badly injured. It is therefore possible that the differences in X-ray susceptibility that occur in different initial materials or in different year-classes of the same strain depend at least to some extent on different seed-size. Thus, for instance, the high X-ray susceptibility in Maja barley as compared with Ymer barley is probably associated with the fact that Maja is smaller grained than the irradiated line of Ymer. The latter showed in 1943 a 1000-grain weight of 45,3 as against 42,5 for Maja.

It must not however be straightaway assumed that large-seeded varieties of a plant species would suffer less from X-ray irradiation than the small-seeded ones. This is shown by, e. g., the Svalöf spring-wheat line Kolben and the Svalöf winter wheat Sammet (Velvet) 0700. Although the latter line is much more susceptible to irradiation, its seeds have a higher 1000-grain weight (Kolben 1942: 33,3, Velvet 0700, 1942: 37,5). The two varieties have, however, a quite different descent and an entirely different physiology. Of course, other conditions than seed-size also influence the susceptibility.

Lastly, it must be emphasized that in theoretical mutation experiments and in methodological investigations it is advisable to use seeds of a certain size, this in order to reduce the variation and create as exact conditions as are at all possible.

*Autopolyploidy.* — In 1941 MÜNTZING examined the susceptibility of diploid and autotetraploid Opal B to irradiation and found that the tetraploid suffers less. As the seeds, on an average, are larger in the tetraploid than in the diploid (in 1941 the 1000-grain weights were

respectively 57,<sub>0</sub> and 47,<sub>1</sub>), the increased resistance is probably partially due to the increased size of the seeds.

Similar results have also been obtained by LEVAN (1944) in flax. Here the strains Hercules and Concurrent, which both rank as fibre-flax, showed a reduced X-ray susceptibility in the tetraploid stage, whereas the tetraploid of Palermo, which is an oil flax, if anything behaved in the opposite way, in any case was not more resistant than the diploid. The X-ray resistance of the diploids is the highest in the large-seeded strain (the 1000-grain weight is 6,<sub>1</sub> gm. in Palermo against 4,<sub>7</sub> and 3,<sub>9</sub> in Hercules and Concurrent). The highest X<sub>1</sub> resistance is shown by the tetraploid in Concurrent, next comes tetraploid Hercules, and lastly tetraploid Palermo (the 1000-grain weights are respectively 6,<sub>6</sub>, 6,<sub>4</sub> and 7,<sub>9</sub> gm.). Probably, therefore, both the absolute and the relative increase in seed-size have an influence on the susceptibility to X-rays.

Results similar to those in barley and flax have also been obtained in white mustard (1944 and 1945), where the tetraploid had a 1000-grain weight of 10,<sub>5</sub> as against 6,<sub>4</sub> gm. in the diploid. The tetraploid stands a higher quantity of X-rays. The critical dose lies between 100.000 and 145.000 *r*. In the diploid it lies at ca. 100.000 *r*.

It is a general experience that polyploid species in a genus stand a higher irradiation than the diploids, in spite of the increase in the number of chromosome disturbances. (See FRÖIER, 1946, for the Svalöf results in wheat and oats.) This is owing to the reduplication of chromosomes and genes.

The investigations hitherto conducted to ascertain the significance of the seed properties for mutation research are only a link in a systematic inquiry into the best conditions for creating valuable mutants. Other factors under investigation have reference to changes in the pH of the seeds (of great importance), protein and carbohydrate contents, influence of chemical substances (colchicine, carcinogenous substances, hormones, etc.).

## II. SPONTANEOUS AND INDUCED MUTANTS IN BARLEY.

### 1. EARLIER RESULTS.

Apart from chlorophyll mutations, which now and then appear in all rather large cultures of barley, several spontaneous mutations have been described in the literature. Dwarfs of various kinds have been observed by VESTERGAARD (1919),

MIYAZAWA (1921), HARLAN and POPE (1922) — in the last-mentioned case the dwarf resembled mut. *densinodosum*, p. 18; cf. also HONECKER (1936) — HOR (1922), HALLQVIST (1923), SCHARNAGEL (1925), and SWENSON (1940). Most of the mutants described here are recessive in relation to the maternal line. The dwarf that was isolated by MIYAZAWA was however dominant, and was fully characteristic already in the heterozygous condition. As homozygous it was but poorly viable. HONECKER (l.c.) observed a dominant or semidominant dwarf mutant. KIESSLING (1912) isolated a mutant characterized by larger foliage, longer internodes of the ears and longer awns, lighter green colour of the leaves. The chlorophyll formation was reduced. This mutant corresponds to certain *viridis* mutants which are viable when homozygous but late in ripening and low in yield.

Among morphological mutants the following are of interest. IKENO (1925) found in his experiments a dominant mutation from six-row to *intermedium* type that was constant generation after generation. TEDIN and TEDIN (1927) described a mutation changing the kernel base. In the progeny of a cross between lines having the *fulsum* character (bevelled kernel base) a form of *verum* type (transversal nick) arose. This mutation took a recessive direction. MIEGE (1927) observed a variant with smooth awns in an experiment comprising two different barley lines — »apparition brusque d'une orge à barbes lisses». It was probably due to »une mutation par perte», although the possibility of spontaneous crossing is not excluded. Both the cultivated lines were however barbed-awned. VESTERGAARD (1928) described the finding of a *zeocriton*-like variant, which had probably arisen through mutation. A transitional form to »hooded» (var. *trifurcatum*) arose in HARLAN's experiment (1931). The mutant was entirely sterile and furnished with pocket-like formations on the lemmas (similar to those in mut. *calcaroides*, p. 26). *Trifurcatum* itself is poor in yield. HARLAN considers it to have arisen as a fairly recent mutation, since in its native country it has not spread notably beyond its true area of origin (Nepal).

In a plant obtained from the cross *H. nutans* × *spontaneum* HUBER (1932) found a bud mutation that caused highly remarkable changes of the phenotype. The basal awns were thickly set with long hairs, and so were the expanded parts of the glumes. Probably this is a case of vegetative mutation confined to the epidermis, for the progeny was normal. HUBER considers that the barbs on the awns of normal barley varieties are homologous with hair formations.

Of great interest from a practical as well as a theoretical point of view is SCHIEMANN's description (1930) of a short-awned recessive mutant from the line »Bethge III». This character, which occurs spontaneously in the East-Asiatic region, was combined with a short, powerful, stiff straw and beautiful, rounded kernels. The mutant, however, had a lower yield than the maternal line. Crossed with »Bethge II» it produced a widely spread malting barley »Bethge XIII», which is considerably stiffer in straw than line II but has the same yield.

IMAI (1935) observed an interesting morphologic mutant in which the leaves of the seedling were leek-like. The root system was poorly developed, and therefore the mutated homozygotes died after a couple of months without producing ears or seeds. It was thus (sublethal or) lethal.

According to a review in Der Züchter 9, 1937, page 102, DICLUS (1936) has isolated as a mutation a f. *triaristatum*, characterized by an awn on each lemma and two awns on each palea. He also described some physiological mutants.

MARTINI and HARLAN (1942) report a series of »barley freaks», some of which were obtained after recombination; others, however, arose as spontaneous mutants. Among the latter are Grandpa, with all colour of the spike lacking (»the beard is white»), »multiflorus», with a large and varying number of flowers at each node, »accordion rachis», with long elastic zigzag curved segments.

GUSTAFSSON has observed two cases of spontaneous morphological mutations: a bud variation with half the ear formed like *distichum* (the mother-line), the other half like *tetrastichum*, and a mut. *scirpoides*. Unfortunately, the six-rowed mutated plant was lost, and therefore its heredity could not be investigated. Respecting mut. *scirpoides*, see page 31.

Waxless mutants form a transition to the physiological group. A spontaneous variant has been described by TOKHTUYEV (1935). So far back as the twenties, however, H. TEDIN (unpubl.) found a waxless mutant out of the Primus barley, which equalled the mother-line in yield.

Physiological mutants have been isolated by POPE (1935) after long selection experiments in various lines of barley. Among 35,000 plants one mutation for increased internodal length was observed (out of Deficiens), one mutation for irregularly spaced nodes (out of Hanna), and one probable mutation for changed internodal length (likewise out of Hanna).

Structural spontaneous re-arrangements in the genome have been demonstrated by SMITH (1941), who in a barley material found one plant with an inversion, which gave bridges at meiosis, one plant with a reciprocal translocation and with 65 % fertility as a consequence, and three trisomic plants with several meiotic disturbances. As early as 1932 EKSTRAND demonstrated the presence of genes for asynapsis.

The mutants that occur spontaneously are mostly lethal or sublethal, though some seem to be fully vital. This applies within the morphological as well as the physiological category. Most of the mutations are recessive in relation to the mother-lines, though at least three cases of dominants are known.

Of works on induced mutations in barley reference must naturally first be made to STADLER's (1930). Besides a large number of chlorophyll mutants he obtained seven non-seedling mutants, two of which were non-glaucous and one dormant (winter-barley like). No dominant mutation was observed.

PISMENKO (1937), cited from Der Züchter, 10, 1938, page 336, produced two mutants by irradiating ears at the time of flowering, one mutant by raying germinating seeds, and two mutants by raying dry seeds. KRAJEVOJ (1939) has given a detailed description of these five mutants: (1) *triaristatum*, which apparently is not identical with the mutant described by DICLUS under the same name but, instead, resembles my »lemma-like glumes», (2) *compactum*, similar to my *erectoides*, (3) *Pismenkovi* with a corkscrew-twisted stem, (4) *non-aristatum* with short early shed awns, sublethal, and (5) *fistulosum*, manifestly much like the mutant that is here named *scirpoides*.

KRAJEVOJ has also carried out certain minor »selection experiments» with these mutants. The best among them seems to be var. *triaristatum*. Its yield has not been tested, though its viability has, in mixed cultures with the original type. The other mutants show poor germinability and reduced viability.

By means of X-raying Abyssinian spring barley LUTKOW (1937 b) produced a mutant of winter-barley type which was recessive to the original form. Nothing

has been reported as to its cold-hardiness. He also obtained a ligule-lacking form (1937 a) and several mutants with changed ear characters (1937 c).

In 1939 NILSSON-EHLE published a preliminary report of the results he had obtained up to then with five to six different erectoid mutants. The mutations combined freely or showed linkage. Two were probably identical. One of the mutants is stated (personal communication) to yield about as much as the mother-line (Golden barley).

According to Der Züchter, 13, 1941, KRAJEVOJ (1940) produced an awnless mutant from an awned variety.

In 1941 (a and b) as well as 1942 GUSTAFSSON described a series of induced mutants in barley, some of which were rather high-yielding. Among forms of morphological interest there are some with changed spike and flower structure. Some ten erectoid mutants had been obtained up to 1941. GUSTAFSSON (1940) submits some data which were intended to indicate a non-random course of the mutation process.

Of great importance is FREISLEBEN and LEIN's find (1942) of an induced mildew-resistant mutant from the line Heines Haisa. With the aid of a specially elaborated technique for mass infection 12,000  $X_1$  offspring (comprising 240,000 seedlings) were tested for their susceptibility to *Erysiphe graminis*. Nineteen of these proved to be partially resistant and were further tested in the  $X_3$  and  $X_4$ . A selected pedigree line entirely resisted infection by the mildew races 1, 2 and 4. In other respects the mutant was completely fertile and differed in no way morphologically from the mother-line. The yield is not yet known. These authors (1943 a, b) have also drawn up some principles for a plant-breeding by means of induced mutations.

BURNHAM (1946) found an induced recessive mutation making the chromosomes unusually long at first meiotic metaphase. Univalents were frequent. The pollen-grains aborted to 15 or 20 %.

## 2. MODEL EXPERIMENTS IN TWO-ROWED BARLEY.

Three complete series of experiments have been carried out in two-rowed barley. In the years 1932—1940 the pure line Golden barley was broken down in different directions. The same thing occurred later (1940—1943) with Maja barley, which is a homozygotized cross-product of Binder barley and Golden barley. From a sister-line ( $b_7$ ) to the so-called Ymer barley (Victory  $\times$  Maja  $\times$  Opal) a series of morphological mutants was raised in 1944—1945.

Golden barley is a pure line that was placed on the market so far back as 1913. It derives from an old land barley of Gotland, was isolated at the turn of the century, and was considered suitable as initial material for the first mutation experiments. During a succession of years Golden barley was the most eminent two-rowed barley in southern Sweden, high-yielding, comparatively straw-stiff and — in its character of a pure line — completely uniform. At the end of the

twenties, however, it had to give way to the Danish strains Kenia and Opal, which combine better quality as malting barley with a higher yield and considerably increased straw strength. Even these, however, were surpassed in yield by the Maja barley, although the latter was not very suitable for malting purposes. The Ymer b<sub>7</sub> barley, finally, stands at about the same level as Maja in yield but has substantially better malting properties.

Since 1939 the experiments have had the following extent (for some of the earlier series, see GUSTAFSSON, 1940):

	No. of plant progenies	No. of spike progenies
1939—1940 Golden barley . . . . .	2630	2630
1940—1943 Maja barley . . . . .	2256	9681
1943—1945 Ymer barley (b <sub>7</sub> ) ..	2010	3345
1939—1945 Controls	626	887

The X-ray doses ranged from 500 to 25.000 r. Dry seed and seed pretreated in different ways were X-rayed.

The mutants produced in the three varieties of barley may be phenotypically divided into three groups: (1) chlorophyll mutants, (2) sterility and lethality mutants, and (3) vital mutants.

#### A. CHLOROPHYLL MUTANTS.

This group of mutants is especially suitable for methodological investigations in diploid plants. Chlorophyll mutations readily arise and can be discovered at seedling stage. The majority are lethal, live as long as the store of nourishment lasts, and then die. They should therefore really be assigned to Group 2 but are conveniently separated from this. A few are subvital, i. e. are able to assimilate, form ears and even ripe kernels, although the ripening is often greatly retarded. In one case there even arose a viable chlorophyll mutant that under certain external conditions seemed to be superior to the original form in yield. In flax LEVAN (1944) has produced a *viridis* mutant that is fully vital also under normal external conditions (p. 85). The mutants can be placed according to their appearance and chlorophyll formation in different subgroups (GUSTAFSSON, 1940; see also FREISLEBEN and LEIN, 1943, as well as FRÖIER, 1946).

The plastids are as a rule normally or subnormally developed in *viridis* mutants of barley, whereas they are small and undeveloped in

*albina* types. Plastids of the *xantha* types occupy an intermediate position. In the *alboviridis* mutants they often develop differently at the apex and base of the leaves (GUSTAFSSON, 1942 b). For oats FRÖIER (l. c.) has observed a somewhat deviating behaviour. The commonest of the mutant types in barley are *albina*, *virescens*, *chlorina*, *virido-albina*. Sporadically *alboxantha*, *xanthalba*, and *tigrina* arise.

#### B. STERILITY MUTANTS.

These mutants form two subgroups.

The *first* comprises such mutants that already have reduced viability in the vegetative stage and consequently poor seed-setting, but also mutants that as a result of altered spike or flower structure are wholly or partially sterile.

Dwarfs with an enfeebled development and partial fertility are not infrequently formed. In the cases examined they remain constant year after year, the ears burst their envelopes incompletely and the seed production is reduced. Narrow-leaved variants with dwarf growth are not uncommon.

In a peculiar mutant that arose from Ymer b<sub>7</sub> in 1945 most of the spikelets were sterile and seed was only developed in the very highest flowers. A similar mutant was described by VESTERGAARD (1928).

One characteristic mutant has repeatedly ramified ears (GUSTAFSSON, 1941, Fig. 7). It is a compact dwarf with numerous nodes and short internodes. Its pollen is normal but in spite of this the plants are entirely sterile. This type of mutant has arisen in Maja as well as Ymer b<sub>7</sub> and seems to be identical with a form described by HARLAN and POPE (1922). It has been given the name of *densinodosum*. SMITH (1939) has found a similar mutant in *Triticum monococcum* (*mi mi*, his Fig. 11), although it was much higher and, in addition, partially fertile.

To this group there also belongs a mutant that has been described by GUSTAFSSON and ÅBERG (1940). The spikelets of the middle-row contained two flowers within each lemma. The long-awned primary lemma looked normal. The two secondary lemmas possessed awns about three-fourths the length of the ordinary ones. Similarly the paleae had awns about 2 cm. in length. Frequently the secondary lemmas grew together. Seeds were never formed, although the pollen looked normal. This mutant type has been found several times. Owing to its complete sterility it has not been specially studied.

SMITH (l. c.) has found several cases of diplontic sterility in *Triticum monococcum*, among others anther-less mutants, where the anthers ceased development before or shortly after meiosis was completed.

The second group of sterility mutants do not produce seed or do so incompletely as a consequence of disturbed meiosis, caused by recessive factors. SMITH observed many such cases in the progeny of X-rayed *T. monococcum*. Sometimes the chromosomes remained unpaired (partial or complete asyndesis), sometimes they were fragmented at first division, in other cases diploid microspores arose, diakinesis was retarded, etc. In some cases pollen was formed but aborted owing to the mature grains lacking starch. The recessives may be vegetatively normal, but also very weak.

#### C. VITAL MUTANTS.

This term includes here such variants as in a vegetative respect do not show any lethal traits and, in addition, are entirely fertile. Vital mutants in barley may grade below, level with or lie above the original line in yield. In the last case they may be called progressive. Even a variant that is inferior to the original line in the latter's proper area of cultivation may occasionally become distinctly superior to it in another climate or another environment, in which case it must also be called progressive.

For the sake of simplicity two subgroups may be distinguished within the group of vital mutants (GUSTAFSSON, 1941 a): (a) *Morphological mutants*: morphological characters are altered in addition to changes in physiological properties. As a rule they segregate sharply in intercrossings or in crossings with the original line; they are qualitative. (b) *Physiological mutants* which, when cultivated alongside the original, can be distinguished from it, but only in properties relating to straw-height, straw-stiffness, earliness, tillering capacity, etc. In the crossings hitherto studied there is no sharp segregation. The  $F_2$ -segregates cannot be divided into two (or three) distinct classes, the phenotypical variation covering the genotypical. Most of BAUR's or TINE TAMME's »Kleinmutationen» belong here.

There is no plain difference between the subgroups. Some winter barley-like mutants, which segregate sharply and are easily distinguished both from heterozygotes and from normal homozygotes by their extreme lateness, form a transition between the two subgroups. The extreme lateness (i. e. the winter-barley character) is rather a phy-

biological property. In the cases studied, however, it was combined with obvious changes in morphology.

#### a. Morphological mutants.

The so-called erectoid mutants are the commonest within the morphological group. In Golden barley ten such mutants have been induced (*erectoides* 1, 2, 4—11). Of these, *erectoides* 8 and 10 have

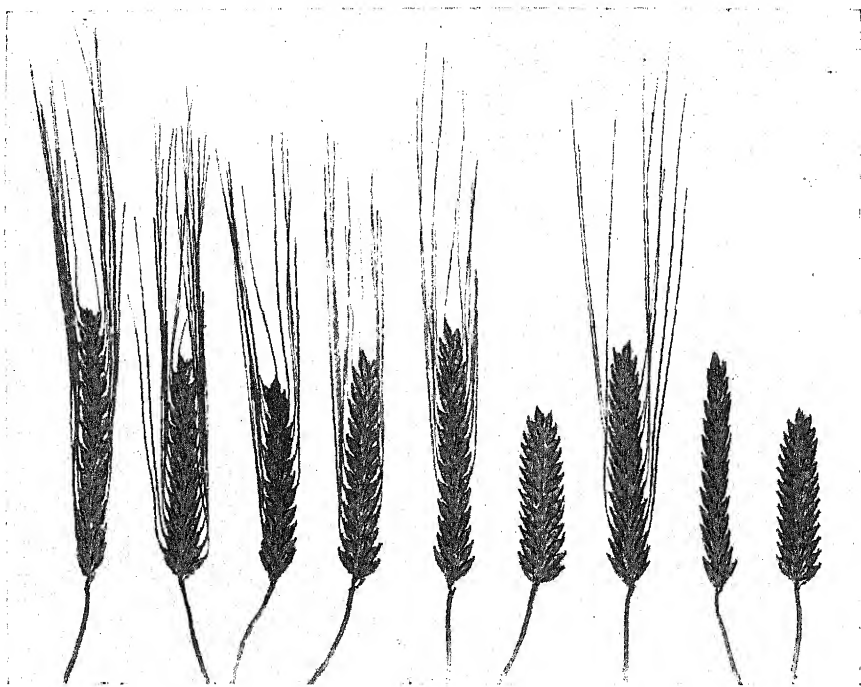


Fig. 1. To the extreme left Golden barley (the mother strain), then the mutants *erectoides* 1, 2, 4, 5, 6, 7, 9, 11.

been lost. (*Erectoides* 3, mentioned in earlier papers, is probably an intermixture.) In Maja barley five mutants of erectoid type have been obtained (12—16) and in Ymer barley, finally, nine (*erectoides* 17—19, 21—24, 27, 28). (The three mutants »*erectoides* 20, 25 and 26», which at their first appearance were placed in this group, fall outside it in spite of having certain characters in common with it.) Something similar may also be said of *erectoides* 16 (Maja). An interesting fact is that NILSSON-EHLE (1939) observed six mutants of erectoid type, arisen out of Golden barley, but no other morphologically deviating

forms. In my own experiments, irrespective of the erectoid types, about 20 vital morphological mutants representing some ten different types have been obtained. Thus, the erectoid type arises oftener, and, as will be shown later, also more readily than most of the other morphological mutants.

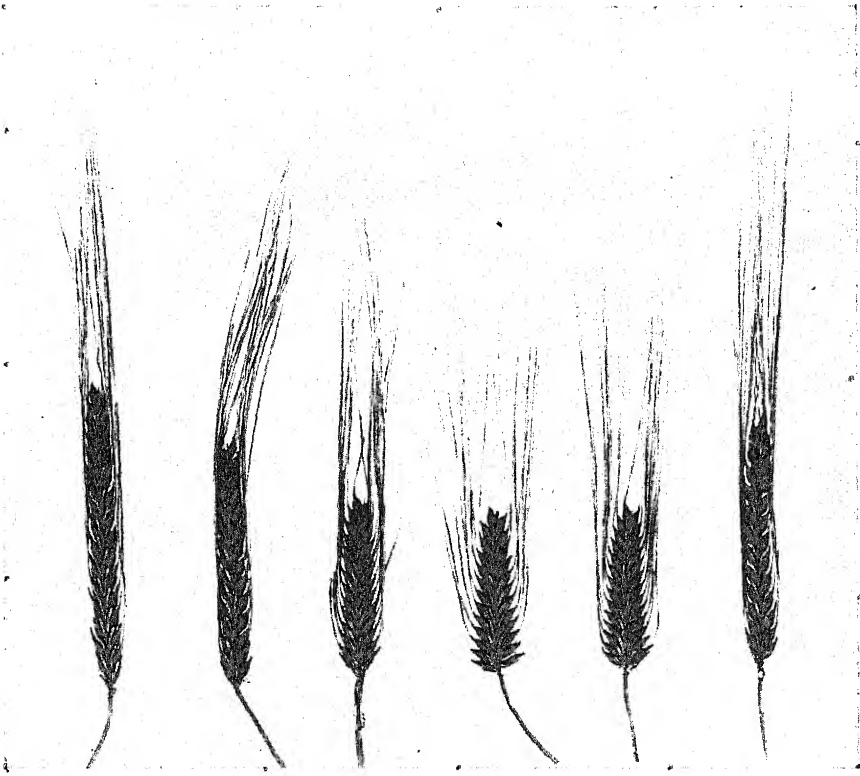


Fig. 2. From left to right: Maja barley (the mother strain), *erectoides* 12, 13, 14, 15, 16.

All erectoid mutants (Figs. 1—3) are characterized by comparatively short, compact ears, often with projecting kernels and awns. A great variety of gradations occur, from subnormal to very short, compressed ears, the last-mentioned resembling those in *zeocriton* barley. The ripe kernels in the two lowest florets often stand straight out from the main axis, thus giving the ears a characteristic appearance, tapering from below upwards. This compact ear type is often accompanied by a short straw. The ears are shortened more than the straws.

In the Golden-barley mutants the relative ear length is 81, in the Maja mutants 77 (both the original lines put to 100). The straw-length amounts to 97 and 93 respectively as against 100 in the controls. Some mutants break this correlation. The most striking is *erectoides* 14,



Fig. 3. From left to right: Ymer barley 40/13 b7 (the mother strain), *erectoides* 17, 18, 19, and a short-awned mutant.

which in spite of its extremely compact ears possesses a substantially longer straw than the Maja barley ( $\text{diff.}_{\text{straw}} = 7,0 \pm 1,6 \text{ cm.}$ ,  $D/m = 4,5$ ;  $\text{diff.}_{\text{ear}} = -2,5 \pm 0,2 \text{ cm.}$ ,  $D/m = 10,3$ ). Thus, the two systems can be changed by way of mutation in opposite directions. *Erectoides* 6 and 11 may also to some extent be ranked as correlation breakers; they

have the most compact ears in the Golden-barley group but the straws are only slightly shorter than in the original line.

The erectoid mutants that have arisen from Maja barley show a considerably stronger variation than those arising from Golden barley. The relative figures for the ear-lengths vary in the Maja mutants between 57 and 97, in the Golden-barley mutants between 70 and 91. The relative figures for straw-length lie in Maja between 70 and 111, in Golden barley between 87 and 101.

The erectoid types possess on an average a lower number of kernels (and with this spikelets per ear) than the original types, but the difference is not considerable. The same applies to the 1000-grain weights. In both groups the mutants fluctuate round their mother-line. Only *erectoides* 16 possesses an appreciably higher 1000-grain weight. The, broadly viewed, constant spikelet numbers and 1000-grain weights, coincident with much shortened ears, obviously explain the projecting appearance of kernels and awns.

In the examined erectoid mutants the straw-strength is always increased. The Golden barley types further differ from the mother-line by upright ears, in the original type they bend downwards. Maja barley and its mutants have upright ears. Even *erectoides* 14, which has acquired a taller straw, shows a distinctly increased straw-strength. The increase is greater, however, in *erectoides* 13, 15 and 16, which are so extremely straw-stiff that under the Svalöf conditions they surpass most other barley lines.

Certain erectoid mutants also deviate from the original lines in earliness. The figures from the yield trials of 1944 may be submitted here. These trials included *erectoides* 1, 7 (from Golden barley), 12, 13, 14, 15, 16 (from Maja barley). *Erectoides* 1, 12 and 13 were this year (as well as other years) about equally early in earing and maturing as the mother-line. *Erectoides* 7 and 15 were distinctly later, two and two to three days respectively. *Erectoides* 14 was a little earlier (two days). *Erectoides* 16 is very remarkable, it ripens extremely quickly. It shoots almost a week before the Maja barley. In the extensive yield trials of 1944 and 1945 it matured about seven days earlier. Whereas in most of the spring barley lines the seedlings remain for a time in a vegetative strengthening stage, in *erectoides* 16 they shoot up direct.

The pronounced pleiotropy shown by many erectoid mutants does not however cease with this. The various barley lines can be divided into an  $\alpha$  or  $\beta$  type, or, as the case may be, a  $\gamma$  or  $\delta$  type, depending on whether they are devoid of teeth on the lateral nerves of the lemmas

or in possession of such (ATTERBERG's system). Golden barley is a  $\beta$  type with 5—7 teeth on each nerve, whereas Maja barley is most closely an  $\alpha$  type, although solitary teeth may be formed. *Erectoides* 1 resembles Golden barley in this respect, while *erectoides* 4 and 5 clearly deviate. They possess on an average about three teeth per nerve. *Erectoides* 9, however, has if anything a higher number, about eight teeth per nerve. Most of the Maja mutants agree with the mother-line, but *erectoides* 14 has more teeth ( $\beta$  type).

The shedding of the awns varies greatly in different mutants. As Fig. 1 shows, *erectoides* 6, 9 and 11 are very apt to shed their awns. Among the Ymer mutants this applies in a specially high degree to *erectoides* 17 (Fig. 3). Attention has been directed on several occasions to the peculiar mutant *erectoides* 16. In this, the erectoid character does not always appear so distinctly; the length of its ear to that of the mother-line bears the ratio 97 : 100, but the lowest grains often project so much that the name can be defended. It deviates from Maja slightly in the dentation of the lemmas but considerably in 1000-grain weight, earliness and straw-strength as well as in regard to its awns, which long before maturing are deeply stained with anthocyanin and are rust-brown in colour.

The examined erectoid types are recessive to the mother-line. In some cases the factors are linked. Free combination gives double recessives of the *zeocriton* type. In several cases, independent in origin, the original line has given identical erectoid mutations.

Among the other morphological mutants mention may here first of all be made of two in which both the glumes at each spikelet were converted into lemma-like formations, each with its awns («lemma-like glumes» 1 and 2). From each spikelet, therefore, there issue three long awns. The mutant has been described by GUSTAFSSON and ÅBERG (1940). It was observed earlier in India and the U. S. A., having presumably also arisen there by recent mutation, and was described by PISMENKO (1937) and KRAJEVOJ (1939) as induced by X-rays. The two Svalöf mutants have been observed in Golden barley.

The glumes have changed in exactly the same way in both cases. The change is probably conditioned by one and the same gene (or by multiple alleles), for the  $F_1$  is identical with the parents and no segregation occurs in the  $F_2$ . One is undoubtedly recessive to the original line and the 3 : 1 segregation is regular. Still, the two mutants are by no means identical. They differ clearly in respect of straw-height and ear-length. They were cultivated in the year 1944 next to each other

and with the original line in the vicinity. One was shorter (diff. =  $-17,8 \pm 1,6$  cm.) and the other taller than Golden barley (diff. =  $=4,2 \pm 1,3$  cm.). Under the conditions of 1944 the two mutants differed in height by not less than 22 cm. In ear-length the mother-line lies midway between the two mutants. Mutant 1 has a shorter ear (diff. =  $-0,44 \pm 0,27$  cm.), mutant 2 has a longer ear (diff. =  $=0,48 \pm 0,28$  cm.). The mutants are thus not definitely separated from the mother-line, though distinctly from each other (diff. =  $0,92 \pm 0,26$  cm.,  $D/m = 3,5$ ). Mut. 1 is richer in anthocyanin than mut. 2; this stands out most clearly in the awns. In addition, it has coloured nerves on the lemmas. Mut. 2 in its turn deviates as regards the teeth on the lemmas; they are certainly not more numerous but are decidedly stronger. Both the mutants have a higher 1000-grain weight than Golden barley.

Of great morphological interest is the circumstance that the lemmas in the lateral rows in both the mutants may be pointed and provided with short awns. In mut. 1 rudimentary lateral flowers with awned lemmas rather seldom occur, in mut. 2 this is commoner and the awns are longer. The very pleiotropic character of the two mutations thus strikes the eye immediately. Especially remarkable is the fact that the straw- and ear-length increases in one mutant but diminishes in the other.

Mut. *intermedium* once arose from Ymer barley. HOFFMANN (see ISENBECK and HOFFMANN, 1942) divided the cultivated barley (*Hordeum sativum*) into four main series: *H. polystichum* (= *H. vulgare*), *H. intermedium*, *H. distichum* and *H. deficiens*, according to the number of fertile spikelets on each node<sup>1</sup>. In *H. intermedium* one, two or three spikelets set mature seed alternatively. The middle row is always fertile and better developed than the side rows. The mutant that has arisen at Svalöf corresponds to the description. According to VAVILOV and ORLOV, similar forms occur spontaneously in Abyssinia and Arabia. Such have also arisen by spontaneous mutation (IKENO). *H. polystichum*, *distichum* and *deficiens* form a multiple-allele series. The factors that control the *intermedium* character lie outside this series. None the less, *polystichum*- and *distichum*-forms, crossed with one another, can give *intermedium*-like segregates in the  $F_2$  and  $F_3$ . According to ISENBECK and HOFFMANN (l. c., p. 148), these arise when

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<sup>1</sup> The system recently introduced by ÅBERG and WIEBE (1946) could not be considered here.

special fertility-factors are combined with the factor for two-rowedness. IKENO's *intermedium* mutant dominated over the six-row type. The mutant described here has not yet been crossed with the original form or with other two- and six-rowed barleys.

Six-rowed (i. e. *polystichum*-) forms have arisen a couple of times, once with certainty out of Golden barley, once out of Ymer barley. Both were first observed in heterozygous form; the side flowers were accordingly more developed than is the case in ordinary two-rowed barley and their lemmas were provided with distinct awns. The former mutant was fertile and gave rise to six-row homozygotes of normal appearance (*tetrastichum* type) but with weak tillering capacity. The other case was associated with complete sterility, and therefore no progeny could be obtained.

A very peculiar mutant, which excellently illustrates how a mutant can alter the morphology of the spikelet, arose out of Ymer barley in 1944 and has been tentatively named mut. *calcaroides* (Fig. 4).

In this mutant the upper part of the lemma arches downwards and forms a sack-like, often even spur-like, recess. The awn is attached to the exterior part of the spur. On account of the shape of the sack the inside of the spikelet comes to lie exposed. In spite of this, self-fertilization seems to predominate, for the progenies from the homozygous calcaroid plants, discovered in the summer of 1944 and surrounded on all sides by normal Ymer  $b_7$  plants, were homozygous throughout in the  $X_4$  as well. The lowest parts of sack and awn are very hairy, sometimes almost hirsute. The barbs on the awns are thinner than in Ymer barley and resemble hairs. The lemmas of the lateral spikelets are larger than in the original type. The kernel base does not slope as is normal in nutans barley but is more sharply inclined (spurius-like). There is considerable variation in the appearance of the sack; the latter may be displaced to the side, upwards or downwards, the recess may be of different depth, sometimes almost vanishing, the awn is differently attached, etc. The pleiotropism thus appears very clearly in this case.

In the progenies that were raised in 1945 from three calcaroid plants the leaves withered away before ripening. Whether the calcaroid characters pleiotropically control this chlorophyll-defect or whether two different mutations have come together by chance, cannot be settled as yet. Calcaroid homozygotes are vegetatively rather feeble in segregating progenies.

This mutant shows striking similarities to a sterile variant that



Fig. 4. *Mut. calcaroides*. Note the spur-like upper parts of the lemmas. This and the mutant in Fig. 5 have arisen from Ymer barley b<sub>7</sub>.

arose in HARLAN's experiments (1931) and that was considered to form a transition to the so-called *trifurcatum*.

In Ymer barley there likewise arose in 1944 a peculiar mutant,

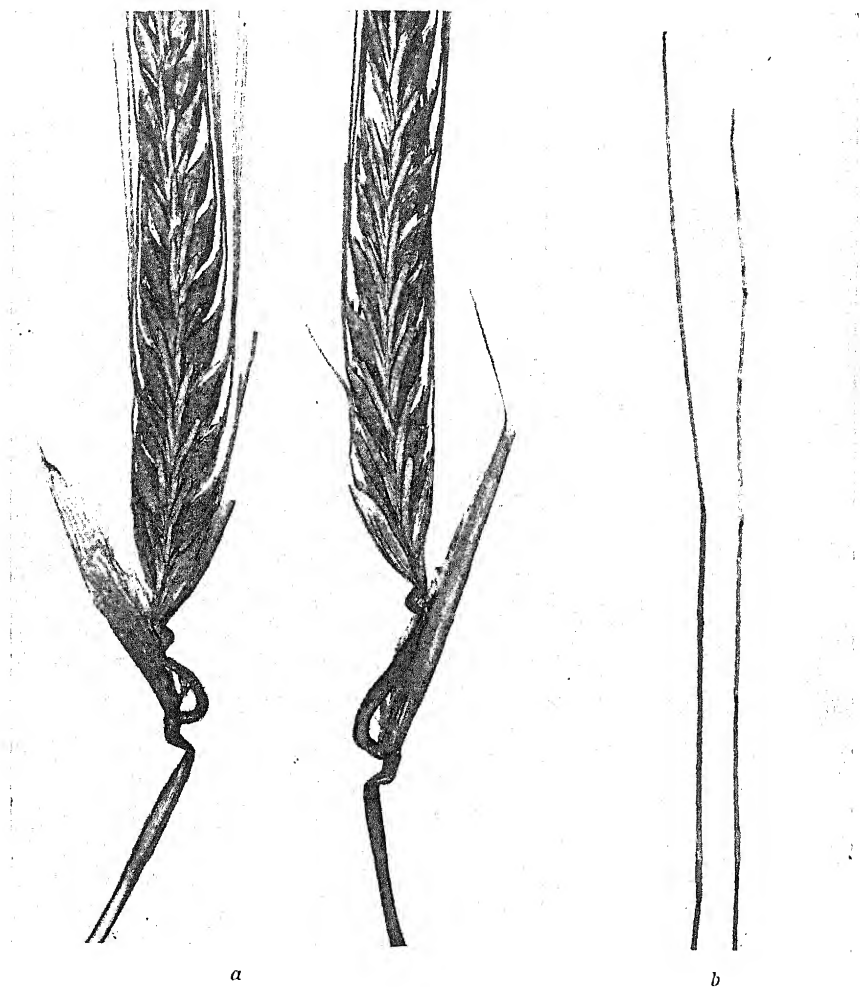


Fig. 5. Mut. *bracteatum*. — *a*. Note the large extra-bracts below the inflorescence, the gradually narrowing bracts in the inflorescence, and the curved culms. — *b*. To the left: the normal appearance of a barley culm (the mother strain), to the right: note the numerous short internodes of the mutant.

tentatively named mut. *bracteatum* (Fig. 5). From extra nodes under the ear there issue bract-like leaves that form a tunic in which the ear is enclosed. In the axils of these bracts are rudimentary, sterile flowers.

From the node under the spikelets there spring successively ever-shorter bracts, and these gradually become scale-like. They are hairy. At the middle of the ear they disappear. The uppermost portion of the culm is curved. In the autumn of 1945 homozygous mutated plants exhibited a peculiar behaviour, folding at the curved section that occurs just beneath the ear and causing this to hang slackly down, though without breaking the culm. The number of nodes on the culms is substantially increased. The two uppermost internodes in normal barley are represented in this mutant by four or five (Fig. 5 b), which become successively shorter.

The homozygous plants are vegetatively robust and very waxy.

From Ymer b<sub>7</sub> there arose in 1944 another peculiar mutant (Fig. 3) that is striking *on account of the short awns* on the lemmas. The mutation is pleiotropic. The culm is shorter, besides being more waxy and much stiffer than in the original line. The auricles are highly anthocyanin coloured. The ear has a weak erectoid character.

From both Maja barley and Ymer barley some mutants have been produced that are characterized *by a peculiarly glaring green colour* and are at the same time entirely or partially waxless. Analysis of the X<sub>3</sub> shows that they are recessive to the normal type and that the heterozygotes segregate simply. Whether all the four mutants are controlled by one or several factors, has not yet been determined. The two Maja mutants are mutually alike. The mother-line, as mentioned earlier, is of the so-called  $\alpha(-\beta)$ -type, one of the bright-green mutants, on the other hand, of  $\beta$ -type. Both have a shorter straw (59,8 and 59,4 cm. as against 65,8 cm. for Maja). The ear-length is practically the same, however, and so is the number of spikelets per ear. The mutant that has given the lowest yield has a substantially lower 1000-grain weight. A spontaneous mutant that is devoid of wax but has not the same peculiar bright-green colour arose earlier in Svalöf (out of Primus barley, H. TEDIN).

In an earlier paper (GUSTAFSSON, 1941 b) reference was made to a mutant out of Golden barley *with half-naked kernels*. At ripening and still more during threshing the lemmas drop from the caryopses. The nakedness is incomplete. This mutant, which was found to have a poor yield, is easily attacked by smut. Certainly it is stiffer in the straw than the original line, but it has considerably smaller and less developed kernels. These possess a high protein content and exhibit a faster germination ripeness than the mother-line.

Two mutants that have arisen out of Golden barley have been

denoted as *winter-barley like* (GUSTAFSSON, 1941). They shoot about one month after the original line and as seedlings remain longer in the vegetative stage. Sown in the autumn, they deviate considerably from



Fig. 6. To the left: two plants of the spontaneous mutant *scirpoides* showing involute leaves. To the right: two plants of Golden barley (the mother strain).

ordinary winter-barley lines in that the seedlings quickly grow out (contrary to the case in the spring) and die early on account of poor cold-hardiness. They are thus characterized rather by extreme lateness than by real winter-barley type (altered photoperiodism?). In one of

them (winter-barley 1) the seedlings are yellowish-green at first and for this reason presumably possess lower assimilation intensity. The other mutant (winter-barley 2) has an almost reed-like appearance on earing. It is tall and robust with very broad leaves. The heights in 1944 of Golden barley, winter-barley 1, winter-barley 2 were respectively  $78,7 \pm 0,9$ ,  $63,5 \pm 0,9$  and  $87,3 \pm 1,7$  cm., and in certain years the differences were still more pronounced. Nevertheless, the ears are considerably shorter in winter-barley 2. The two mutants are more liable to shed their awns than the mother-line and have a lower 1000-grain weight than the latter. Besides these characters the two mutants also deviate in respect of number of teeth on the lemmas, which is considerably lower than in Golden barley itself. Winter-barley 2 is further characterized by the fact that certain lateral flowers have pointed lemmas, winter-barley 1 by coloured nerves on the lemmas.

Finally, mention must be made of a morphological mutation which, although not having arisen in the progeny from X-rayed seeds, appeared in the  $F_2$  of the cross *erectoides* 7  $\times$  *erectoides* 6, both X-ray mutants. It has been named mut. *scirpoides* (Fig. 6). Before the earing the erect leaves, which enclose the spike, give a peculiar appearance to the mutant. The leaves are involute, subulate and yellow at the apex. Possibly the mutation has arisen as a result of chromosomal rearrangements which were induced at the origin of the parents and which have caused pairing disturbances in the double heterozygote (?). Although both the parent lines have been cultivated on a large scale, neither of them has given mutations in its progeny. Three of seven  $F_2$  progenies segregated in both the erectoid and the scirpoid character. The reciprocal combination, which consisted of ten  $F_1$  plants, did not give any scirpoid types. The scirpoid phenotype is controlled by a Mendelian factor which seems to segregate independently of the two erectoid factors. It has admitted of being transmitted without difficulty to normal nutans barley (*AABB*), the two single recessives (*Aabb* and *aaBB*) of erectoid type as well as to the double recessive (*aabb*) of the *zeocriton* type.

#### b. Physiological mutants.

The physiological mutants, like the morphological, affect all organ systems, although they chiefly have a quantitative character and do not deviate from the maternal line so much that progenies from crosses can be assorted into distinct classes. None the less, in this group there

are also mutants which, cultivated side by side with the original line, differ considerably.

Outwardly the most deviating is perhaps a mutant out of Maja barley (44/98) with a greatly shortened straw. At measurements in 1944 the straw was reduced by 40 %, from 64,2 to 38,8 cm. ( $D/m = 30,2$ ). The ear has likewise become shorter, though not in the same proportion, the shortening here being from 7,9 to 6,7 cm. Still, the difference is statistically significant ( $D/m = 7,0$ ). The shortening is due to there

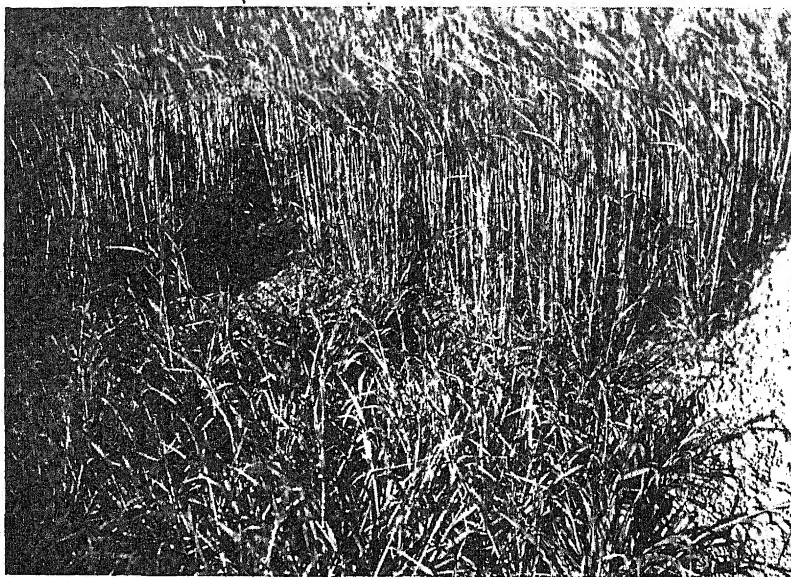


Fig. 7. Winter barley-like mutant (No. 2). In the back-ground Golden barley. Photographed on July 9th, 1941.

being fewer spikelets, not to shorter internodes as in the erectoid mutants. A considerably lower 1000-grain weight falls to the mutant than to the mother-line (35,7 against 43,2). There is good tillering capacity. The mutant belongs to the  $\alpha$  type like the mother-line but, like this, has one or two solitary teeth on the lemmas.

The least changed in appearance of the isolated mutants, (44/23), deviates in leaf-breadth and 1000-grain weight as well as — and above all — in malting qualities (p. 73). In other respects it seems to be identical with the mother-line (Golden barley). Its morphological characters are the same, and so are its straw-height, straw-strength, ear-length and earliness. In yield it shows about the same level as

Golden barley. The leaf-breadth<sup>1</sup> in the mutant was 11,7 mm. in 1944 as against 10,1 in Golden barley, the difference being statistically significant ( $D/m = 7,3$ ). Somewhat shorter leaves mark the mutant ( $\text{diff.} = 1,77 \pm 0,61$  cm.). In the yield trials of 1943—1945 the average 1000-grain weight amounted to 44,4 against 40,4 for Golden barley. This mutant arose in the same pedigree progeny as the extremely broad-leaved winter-barley like variant (No. 2).

In these two cases the mutants differ distinctly from the maternal



Fig. 8. To the right: an extremely straw-stiff and bushy mutant (44/3), to the left: a straw-weak mutant (44/4). Yield experiments (1942).

line only in one external character. Other mutants, however, deviated in many ways. Especially interesting is a type that was obtained in 1939 from Golden barley (44/3). It differs much from all barley lines growing at present at Svalöf. The seedlings early become very bushy and at the same time extremely waxy. The ear does not slope as in Golden barley but is erect, and the culm is considerably shorter. A most remarkable feature is the extreme straw-strength, in Svalöf evaluated in 1944 and 1945 at 9,3 as against 6,2 for Golden barley. This relation has held year after year, even in other areas of cultivation. In 1945

<sup>1</sup> In this as well as other cases the leaf-breadth was measured three weeks after earing on the second leaf of the main shoot, counting from the top.

such large quantities of rain fell in the month of July over the area in which the branch station of Västernorrland is situated that even the otherwise straw-stiffest strains were felled to the ground. Only this mutant kept upright. The ripening time is about the same in the mutant as in Golden barley, if anything shortened by one or two days. Both mutant and mother-line belong to the  $\beta$  type. The extreme straw-strength is counteracted by two bad properties, a low 1000-grain weight and an inferior malting quality. Probably the low 1000-grain weight is due to the fact that the awns drop off to a large extent before ripening, just at a time when they are still able to assimilate. In yield the mutant lies at practically the same level as the maternal line. This interesting mutant reveals one of the disadvantages of the X-ray method: while good »progressive» properties can certainly be induced, changes often arise that reduce the value of the mutant.

Increased vegetative development, specially *higher straw and retarded ripening*, are often combined. Only seldom, however, are the changes so extreme as in the winter-barley like type 2 (p. 31). The culm may be some centimetres higher than in the mother-line, earing and ripening may occur a couple of days later.

One of the oldest mutants in cultivation, (44/2), differs from Golden barley chiefly by higher straw (in certain years up to ten centimetres) and two to five days later maturation. In spite of the higher straw the straw-strength is about as good as that in the mother-line. The 1000-grain weight is generally somewhat higher than in Golden barley, amounting for the five years 1940—1944 to 44,<sub>9</sub> as against 43,<sub>9</sub>. The yield exhibits a tendency to rise during dry summers and to fall during rainy ones (p. 64). Thus, the mutant appears to be more resistant to drought than Golden barley.

Mutants with increased straw-height and lateness have been encountered several times in both Golden and Maja barleys. These two characters are often combined with *increased leaf-breadth, lighter green colour, reduced waxiness*.

The *straw-strength* not so infrequently changes, in a positive as well as a negative direction. As was earlier mentioned, most of the erectoid mutants possess increased straw-strength. A very straw-stiff physiological mutant that arose out of Golden barley has been previously mentioned (the waxy mutant 44/3). Such variants have also been produced from Maja barley. A Maja mutant (44/18), which has now been tested in yield trials for two years and is equal to the maternal line in yield, differs by high straw-strength (8,<sub>2</sub> against 7,<sub>5</sub>), somewhat

higher straw (diff. =  $5,5 \pm 1,2$  cm.,  $D/m = 4,5$ ), greater waxiness, somewhat higher 1000-grain weight and, above all, increased earliness in earing as well as ripening (2—4 days). In another mutant (44/30) the straw-strength increased still more strikingly (8,9 against 7,3). The straw-height, 1000-grain weight and earliness were in this case exactly the same. On the other hand, the colour of the ripe kernels was different, more yellowish, the kernels as well as in fact the whole plant not being so rich in anthocyanin.

On several occasions mention has been made of mutants that combine an increased or decreased 1000-grain weight with other changes. Especially remarkable among these *large-kernelled mutants* is a type out of Golden barley (44/7) with an average 1000-grain weight for the years 1941—1945 of 46,4 as against 41,2 for the original line, i. e. an increase of 13 %. This mutant also differs in other respects. The colour of the kernel on ripening is more yellowish-brown, although in other ways the mutant is quite as rich in anthocyanin as the mother. It also has somewhat broader leaves. In straw-height, straw-strength, earliness, etc. it deviates only slightly and has the Golden-barley habit in other respects. The malting properties are improved. Similar variants have also been obtained from Maja barley. One of these (44/91) possessed in the spring of 1945 a 1000-grain weight of 52,0 gm. as against the 46,1 gm. of the Maja control. As in the preceding case, the kernel colour had changed to a distinctly yellowish-brown tone. The straw was substantially higher (diff. =  $9,8 \pm 1,3$  cm.,  $D/m = 7,7$ ) and the ear was considerably longer (diff. =  $1,5 \pm 0,2$  cm.,  $D/m = 7,2$ ) than in the mother. Simultaneously, however, the straw-strength had fallen considerably. The kernels belong to the *a* type.

Several mutants with *increased earliness* have been dealt with in the previous text. The most interesting from a practical point of view is *erectoides* 16 (p. 23), which ripens at Svalöf quicker than other Scandinavian barley lines and is as early as the early Australian and Egyptian types included in the assortment. (It differs entirely from these, morphologically among other things by the anthocyanin-coloured auricles.) Among the physiological mutants certain variants from Golden barley are earlier than the maternal line, although only to the extent of one or two days. One of them, the waxy mutant (44/3), has already been discussed. Reference has also been made earlier to one of the early physiological mutants (44/18) out of Maja barley.

The results show that changes are more liable to occur towards increased lateness than in the opposite direction.

In GUSTAFSSON (1941 b) some results relative to the process of *germination ripeness* have been discussed. Changes in this character were attended by alterations in various morphological and physiological characteristics. No selection for solely accelerated or delayed germination ripeness has been made.

Respecting properties such as *protein and starch content* as well as *malting properties*, see page 69.

### III. THE FERTILITY OF THE MUTANTS. SYNTHETIC STERILITY.

The vitality of a mutant is determined to an essential extent by its seed production. This in its turn is bound up with the ear fertility, number of spikelets on an ear, and the tillering capacity. The germinability is also of importance.

The direct *fertility* has been determined for eight morphological mutants from Golden barley — all belonging to the *erectoides* type. This was done by counting the number of unfertilized florets and number of developed kernels on the main straw of 25—75 plants taken at random. The fertility of such commercial varieties as Golden, Maja and Ymer barleys usually amounts to 96—98 %. On this occasion Golden barley showed a fertility of 98,0 %. The lowest value for any mutant amounted to 96,1 % (*erectoides* 2) and the highest to 98,7 % (*erectoides* 7). The D/m varied between 0,02 and 1,87. Thus, none of these mutants have a notably reduced fertility. Where sterility occurs, it does not amount to more than a few per cent. The same applies to the great majority of pure-bred mutants that have been tested in yield trials<sup>1</sup>. As a matter of fact, during the last few years only such mutants have been taken out as, judging by ocular inspection, are fertile. Hence a conscious selection works here in the service of the practical work.

*The number of kernels* produced by an ear is determined not only by the sterility but also by the number of spikelets (florets). If no sterility occurs — as in the above-mentioned erectoid mutants — the number of kernels is determined by the number of spikelets that have

<sup>1</sup> Five morphological mutants out of Ymer b<sub>7</sub> have been examined in the X<sub>4</sub> (1945) in regard to their fertility. The maternal line showed an average fertility of 96,0 %. *Erectoides* 19, »bright-green» 4, and »short-awned *erectoides*» gave respectively 96,6, 98,1 and 94,4 %. Two other mutants (*erectoides* 17 and 18) contained, however, a number of plants with reduced fertility, the average figures being 79,9 and 85,3 %. The material was as yet not entirely homozygotized for structural re-arrangements.

been formed. Even the commercial strains exhibit differences in this respect. For instance, on analysis in the summer of 1945 Maja barley gave an average of 26,4 spikelets per ear, Golden barley an average of 24,2. The difference is  $2,3 \pm 0,7$ ;  $D/m = 3,3$ . In one trial in 1944 the number of ripe kernels per ear in the same strains amounted to 26,8 and 24,2 (difference =  $2,6 \pm 0,4$ ;  $D/m = 6,8$ ). The higher yield of the Maja barley compared with Golden barley largely depends on a greater number of spikelets.

In the examined mutants a clear group difference is observed according to their descent. Ten morphological mutants out of Golden barley showed an average of 23,9 kernels per ear against 24,2 for the maternal line, seven morphological and fifteen physiological mutants from Maja barley gave 26,1 kernels per ear against 26,8 for Maja itself. The kernel numbers of the two mother-lines show a ratio of 100 : 111, those of the two mutant groups, 100 : 109 — hence a strict parallelism<sup>1</sup>.

The individual mutants differ by the following  $D/m$  values from the mother strains:

	Fewer kernels per ear					More kernels per ear				
$D/m$ .....	— 3	— 2	— 1	— 0	— 1	— 2	— 3	—		
Number of cases .....	8	1	6	4	4	2	4	3	= 32	

Thus, in every *fourth* vital mutant examined the number of ripe kernels per ear has been so reduced that it definitely differs from that of the maternal line ( $D/m = 3$ ). In every *eleventh* mutant it has increased in a corresponding degree. Negative as well as positive mutants may thus arise. In all cases, i. e. even in those with high  $D/m$  values, there has been only a slight decrease (or increase) in the absolute number of kernels. Usually the change is one of merely two or three kernels per ear, as the following figures show. One mutant, however, falls quite outside the frame. (This is the partially sterile mutant 44/32 mentioned in the foot-note):

	Decrease										Increase									
Change in kernel number .....	7	— 6	— 5	— 4	— 3	— 2	— 1	— 0	— 1	— 2	— 3	— 4	— 5	— 6	— 7					
Number of cases .....	1	—	—	1	5	2	10		6	5	2	—	—	—	—	= 32				

The eleven mutants that showed the  $D/m$  values of 3,0 and more deviated on an average by three kernels per ear from the mother-lines.

<sup>1</sup> If one of the Maja mutants, namely (44/32), which is partially sterile is left out of account, the agreement will be still better, viz. 100 : 110.

Although the direct sterility has only been determined on eight of these 32 vital mutants, it can be unhesitatingly asserted that the great majority have a fertility equal to that of the maternal line, only one or two per cent of the flowers failing. There can be no question of any strong gamete or embryo lethality.

In the summer of 1945 *the number of spikelets (florets)* per ear in some thirty mutants was determined by direct counts. The mutants studied were such as had been included for one or several years in the yield trials, and were thus more or less valuable from a practical point of view.

	Lower number of florets							Higher number of florets						
Change in number of florets	5	4	3	2	1	0	—	1	2	3	4	5		
Number of cases .....	—	—	3	1	11	9	4	—	—	1			= 29	

Only one mutant (44/9 from Golden barley) differs notably from the mother-line. The difference is significant in this case ( $D/m = 6.8$ ). In other respects this mutant deviates by its pale-green tone of colour, broad leaves, increased culm- and ear-length and its lateness. Otherwise the Golden barley type has been closely kept.

Seventeen mutants, which were examined both as regards kernel number (1944) and number of spikelets (1945), showed a positive, significant correlation between the two properties. This confirms the view that the raised (or reduced) kernel number is mainly due to the fact that the number of spikelets has risen (or fallen).

*The tillering capacity* of a commercial strain, as in a mutant, has a great influence on the yield. Twenty-two mutants out of Maja barley showed in 1944 the following  $D/m$  values for the differences from the mother-line (25—75 plants taken at random):

	Lower tillering capacity						Higher tillering capacity					
$D/m$ .....	—	3	2	1	—	0	—	1	2	3	—	
Number of cases .....	—	3	1	4			4	7	2	1	= 22	

One mutant falls entirely outside the variation of its mother-line (44/89). Probably some of the variants that have  $D/m$  values between 2 and 3 are also genetically distinct from the maternal line. On page 37 it was pointed out that every eleventh mutant has an increased kernel number, counted per ear. According to the above group of figures, every *twenty-second* mutant has a significantly richer tillering

capacity. No mutant has yet been observed that combines positive deviations (by  $D/m > 3$ ) in both the characters. But the mutant 44/89 just mentioned will probably prove on closer examination to be so constituted, the difference from the maternal line amounting in tillering capacity to  $1,00 \pm 0,33$  ( $D/m = 3,03$ ) and in the number of kernels per ear to  $0,98 \pm 0,37$  ( $D/m = 2,65$ ). In three cases (*erectoides* 12 and 14 as well as 44/98) the tillering as well as the kernel number was reduced. The yield for *erectoides* 12, however, ranks level with that of the Maja barley itself.

As regards the *germinability of the seeds* it may be said in general that this is not reduced in any of the mutants that have been tested in yield trials. At the sowing in 1945 the germinability was 99 % in seven specially examined morphological mutants, and likewise 99 % in twelve physiological mutants. The two maternal lines Golden barley and Maja barley showed 98 %. In the laboratory experiments of 1945, especially arranged to elucidate the germinating ability, the germination figures for the same mutants were 97—100 %. Several mutants show a retarded germination ripeness in certain years (p. 36). But this change is of an enzymatic nature and does not signify any lethality.

In his paper of 1926 HALLQVIST showed that dihybrids between different chlorophyll mutants often have disturbed segregation numbers in the  $F_2$ , although female gametes and zygotes are only exceptionally lethal. If germinability is lowered, the fall is usually quite inconsiderable (in the most pronounced dihybrid case 6,6 %; monohybrid and constantly green plants possessed a zygote lethality of 2,2 %). The same applies to the seed-setting (in the same dihybrid 5,2 % empty florets occurred against 1,7 % in the control plants). This synthetic lethality constitutes the lowest degree of sterility.

A similar form of synthetic lethality, or rather synthetic sterility, arises if different induced erectoid mutants are crossed with one another. The erectoid character (dense, compact ears) is recessive in relation to the nutans type (sparse, slack ears). Monohybrids usually segregate in the proportion of 3 : 1. Dihybrids give in the  $F_2$ , unless linkage occurs, 9 normals : 6 *erectoides* (single recessives) : 1 *zeocriton* (double recessive). In monohybrids the fertility falls only slightly, whereas in dihybrids it shows a steep fall. This behaviour has been clarified with particular care for three erectoid mutants, all of which had arisen from Golden barley.

Golden barley	P-fertility	97,0 %	25 plants, 25 ears
<i>Erectoides</i> 1	»	98,0 %	25 » 25 »
<i>Erectoides</i> 6	»	96,2 %	25 » 25 »
<i>Erectoides</i> 7	»	98,7 %	25 » 25 »
<hr/>			
<i>Erectoides</i> 1 × Golden barley	$F_1$ -fertility	almost normal, not accurately analysed	
<i>Erectoides</i> 6 × Golden barley	»	91,6 %	20 plants, 80 ears
<i>Erectoides</i> 7 × Golden barley	»	89,9 %	16 » 79 »
<hr/>			
<i>Erectoides</i> 1 × <i>erect.</i> 6	»	80,7 %	8 » 31 »
<i>Erectoides</i> 6 × <i>erect.</i> 1	»	79,2 %	16 » 68 »
<i>Erectoides</i> 1 × <i>erect.</i> 7	»	59,4 %	12 » 44 »
<i>Erectoides</i> 7 × <i>erect.</i> 1	»	55,0 %	29 » 129 »
<i>Erectoides</i> 6 × <i>erect.</i> 7	»	68,6 %	10 » 48 »
<i>Erectoides</i> 7 × <i>erect.</i> 6	»	72,9 %	7 » 61 »

The crosses with Golden barley give a distinctly even if weakly reduced fertility (6 or 7 % below the mother-line). The sterility is far more conspicuous in crosses between the erectoid types themselves, especially in the cross *erectoides* 1 × *erectoides* 7 and reciprocally. In all cases the reciprocal crosses behave identically. No maternal effect is thus seen. These results suggest that the irradiation together with the distinct »gene-change» that results in the erectoid type has also brought about other changes in the genome, e. g. minor inversions and translocations. In homozygous condition these structural re-arrangements exercise no lethal effects and therefore probably do not constitute deficiencies. In heterozygous state — after crossing with the original line — they reduce the fertility somewhat, presumably owing to small meiotic disturbances. If such aberrations are brought together from different mutants independent of one another, their effect is added and the sterility increases considerably. According to this explanation, the 50 % sterility of *erectoides* 1 × *erectoides* 7 is not due to a single reciprocal translocation but to an accumulated effect of genomic re-arrangements. This behaviour resembles what MÜNTZING (1938) has demonstrated for line crosses within *Galeopsis*.

The pronounced pleiotropic effect of the morphological mutants probably depends in several cases on the interaction between an altered gene, often with strong effect, and a number of modifiers, which in their turn may be genic mutations but are probably often associated with structural re-arrangements (position or »pattern» effects, etc.). X-raying strikes and changes the cell nucleus at many points. But complex changes may also arise in association with spontaneous mutations. The additive effect that these show at crosses is certainly less

than that of the X-ray mutations but is nevertheless unmistakable in several cases.

#### IV. MUTATION FREQUENCIES IN BARLEY.

##### 1. SPONTANEOUS MATERIAL.

Hardly any exact data on the frequency of spontaneous barley mutations are to be met with in the literature. HALLQVIST (1924) found eight plant progenies of about six thousand to be heterozygous for various chlorophyll mutants. His material mainly consisted of progenies from crosses. In pure lines of Scandinavian commercial varieties, e. g. Golden barley, NILSSON-EHLE (unpubl.) has estimated that a new spontaneous chlorophyll mutation appears in the progeny from about ten thousand plants. PETO (1937), on inspection of field material, found 149 mutants among 1,390,000 normal plants (i. e. about one in 10,000). Since the mid-thirties the author has made notes on the progenies from all X-rayed seed as well as on all the material planted as controls. Several spontaneous chlorophyll mutants have been observed during the period covered.

In Golden barley progenies from 8,279 individual plants were specially studied. At least four plants were heterozygous for spontaneous mutations, that is 4.8 per 10,000. No chlorophyll homozygote was observed in the first generation.

In Maja barley two plants out of 2,256 proved to be heterozygous for chlorophyll mutations, i. e. 8.9 per 10,000.

In Ymer  $b_7$  barley, finally, one heterozygote was found among 707 offspring, 14.1 per 10,000. (During a field examination in 1944 one *albina* recessive was observed among 4,600 seedlings.)

The different mutant types (*albina*, *alboviridis*, *viridis*, etc.) arise spontaneously in about the same proportions as after X-ray treatment. In X-ray experiments the *albina* mutants usually compose 50—60 % of all mutants. Of twelve spontaneous mutants, five (42 %) were *albina*, two (17 %) were *alboviridis*, and five (42 %) were *viridis* types.

Some experiments have been carried out with the object of clarifying the frequencies of mutations in heat-treated and aged seed material. In series from 1939, where seed material from Golden barley consisting of dry as well as of water-soaked seed had been gradually given a temperature of 80° C. for 24 hours, three to four mutants appeared in 473 progenies. This represents a heterozygote frequency of

63—85 per 10.000, i. e. an increase fifteen times that shown by the normal material. PETO (l. c.) also found a substantial increase.

In 1939 plants were also raised from aged seed (stored with a 15 % water-content, material from 1932—1935). The progenies from 697 such plants contained five mutants, i. e. 72 heterozygotes per 10.000 plants or a fifteen-fold increase in the number of heterozygotes.

The following table shows the results obtained:

Golden barley, pure line . . . . .	4	heterozygotes in 8279 plants, 4,8 per 10.000
Maja barley, old cross variety . . . . .	2	» » 2256 » 8,9 » »
Ymer barley b <sub>7</sub> , new cross variety ..	1	» » 707 » 14,1 » »
HALLQVIST, 1924, cross material ....	8	» » 6000 » 13,3 » »
<hr/>		
Golden barley, heat-treated seed ....	3	» » 473 » 63,6 » »
Golden barley, aged seed . . . . .	5	» » 697 » 71,7 » »

The total frequency of chlorophyll mutants under normal conditions corresponds to the effect in the X<sub>2</sub> of 40 *r*-units (Golden barley), 70 *r* (Maja barley) and 110 *r* (Ymer b<sub>7</sub> barley). Heat-treated and aged seed give mutant frequencies corresponding to about 500 *r*.

## 2. INDUCED MUTATIONS.

*Chlorophyll mutation.* — The number of chlorophyll mutants formed in different cereals after X-ray treatment was first determined by STADLER. In his work of 1929 he puts the mutation frequency in barley at  $4,9 \cdot 10^{-6}$ , in the diploid *Avena brevis* and *strigosa* at  $4,1 \cdot 10^{-6}$  and  $2,6 \cdot 10^{-6}$ , and in the diploid *Triticum monococcum* at  $10,4 \cdot 10^{-6}$ , all calculated per *r* unit and *ear* progeny in the X<sub>2</sub> generation.

In 1940 GUSTAFSSON reported the frequencies for different mutation types in barley. Calculated per *ear* progeny the total mutation frequency in the X<sub>2</sub> generation is about  $4 \cdot 10^{-6}$  for every *r* unit.

FREISLEBEN and LEIN (1943 b) gave a series of valuable data, likewise derived from X<sub>2</sub> analyses. They find that the mutation frequencies do not rise proportionally to the amount of X-rays (Fig. 9). In the most extensive series (from the year 1941) the absolute frequencies increase up to a dose of 8.000 to 10.000 *r* in order to fall thereafter. If the frequencies are calculated per *r* unit, they are found to fall continuously from  $18,4 \cdot 10^{-6}$  at 4.000 *r* to  $5,6 \cdot 10^{-6}$  at 14.000 *r*. In the second series (from 1942) the absolute frequencies increase rapidly up to 15.000 *r* but then fall again. Calculated per *r*, the values lie at

about the same height. In both cases the frequencies have been calculated per *plant* progeny<sup>1</sup>.

The number of *albina* and *xantha* mutants in Maja barley has been determined in the  $X_3$  generation after irradiation with six doses between 2,500 and 25,000 r (1941—1942). Just as in FREISLEBEN and

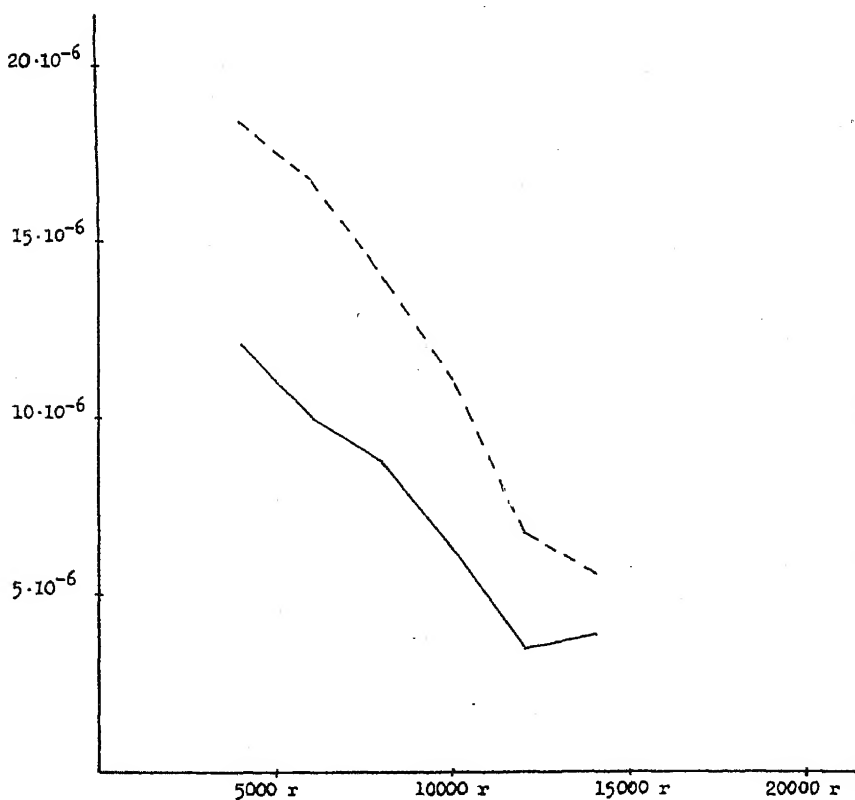


Fig. 9. The frequency of *albina* mutants, calculated per *r*-unit and plant progeny (lower curve). The upper curve shows the frequency of all chlorophyll mutants, calculated per *r*-unit and plant progeny.  $X_2$ -material. (After FREISLEBEN und LEIN, 1943 b, series 1941.)

LEIN's first series, there was a steep fall in the mutation frequencies per *r* unit with increased X-ray dose (Fig. 10). On an average the  $X_1$  plants have developed three to four ears per plant, and hence the frequencies can also be calculated per ear progeny.

<sup>1</sup> The two authors cultivated their  $X_1$  plants under very favourable conditions. The plants therefore tillered copiously, giving up to 15 ears per plant.

During the years 1943—1944 a third analysis was carried out, this time in Ymer barley  $b_7$  (10.000—25.000  $r$ ). Only one ear (the principal ear) from each  $X_1$  plant was sown. The mutation frequency, calculated per  $r$  unit, falls successively and reaches its minimum at 25.000  $r$ , the same result, accordingly, as was obtained in Maja.

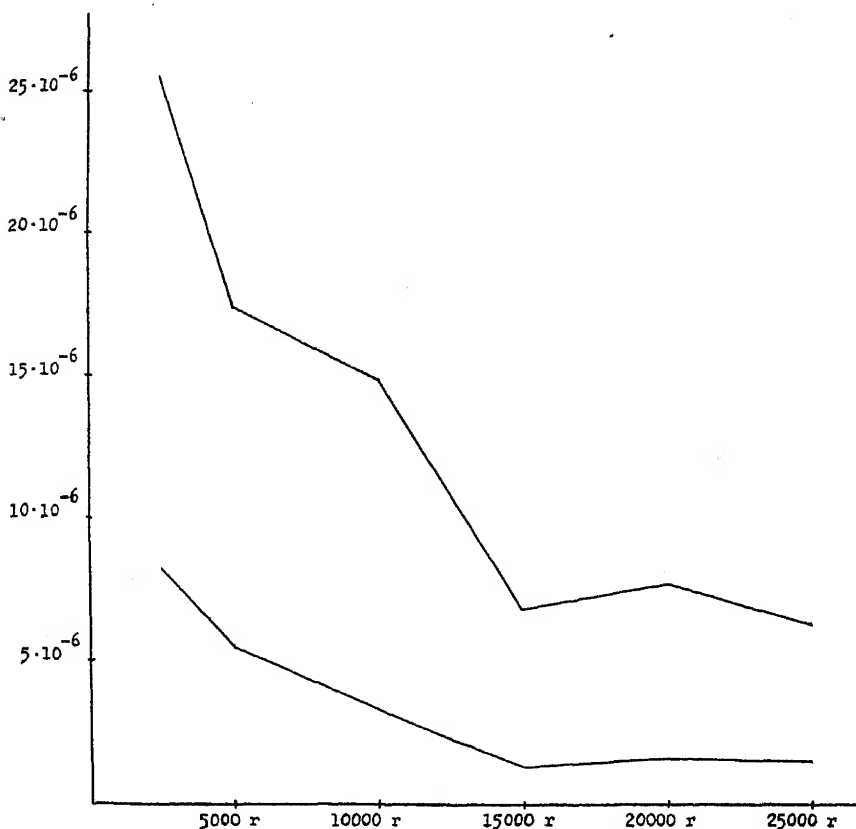


Fig. 10. The frequency of *albina* mutants in Maja barley, calculated per  $r$ -unit and plant progeny (upper curve), per  $r$ -unit and ear progeny (lower curve).  $X_a$ -material.

The absence of proportionality between dose and mutation frequency may be due, as FREISLEBEN and LEIN also point out, to different concurrent circumstances, viz. (1) a saturation effect, that is to say, at high doses a certain mutation type arises several times without its being possible to distinguish the different mutation cases from one another, (2) an S-shaped dose curve; certain mutations and mutation types are not one-hit phenomena but derived from structural re-arrangements, (3) selective conditions during ontogenesis, the most lethal changes being eliminated in favour of undamaged chromosomes and genes, in which connexion the fact also

TABLE 4. *The mutation frequencies of various types of chlorophyll mutations in barley.*

	P l a n t   p r o g e n i e s				E a r   p r o g e n i e s			
	FREISLEBEN and LEIN, 1943		GUSTAFS- SON, 1940	Maja barley	Ymer barley	GUSTAFS- SON, 1940	Maja barley	Ymer barley
	I. 4.000— 14.000 <i>r</i> $X_2$	II. 8.000— 20.000 <i>r</i> $X_2$	5.000— 10.000 <i>r</i> $X_2$	2.500— 25.000 <i>r</i> $X_3$	10.000 <i>r</i> $X_3$	5.000— 10.000 <i>r</i> $X_2$	2.500— 25.000 <i>r</i> $X_3$	10.000— 25.000 <i>r</i> $X_3$
<i>Albina</i> .....	$7,4 \cdot 10^{-6}$	$7,7 \cdot 10^{-6}$	$6,2 \cdot 10^{-6}$	$13,0 \cdot 10^{-6}$	$12,0 \cdot 10^{-6}$	$\pm 2,1 \cdot 10^{-6}$	$3,4 \cdot 10^{-6}$	$4,6 \cdot 10^{-6}$
<i>Xantha</i> .....	0,8	0,5	0,9	3,2	2,0	$\pm 0,3$	0,3	0,2
<i>Albovidis</i> .....	1,4	1,0	3,0	—	5,0	$\pm 1,0$	—	0,9
<i>Viridis</i> .....	2,6	5,4	2,3	—	9,0	$\pm 0,8$	—	1,2
All mutations .....	12,1	14,7	12,7	—	30,0	$\pm 4,2$	—	7,2
N .....	802	936	103	139	30	103	139	90
								30
								4,0 · 10 <sup>-6</sup>
								0,7
								1,7
								3,0
								10,0

(All values calculated per *r*-unit.)

has its effect that small seeds are more liable to be killed than large ones and the latter give lower mutation frequencies.

According to the summary given in Table 4, the total frequency of chlorophyll mutations in the  $X_2$  generation can be calculated at  $\pm 13 \cdot 10^{-6}$  per X-ray unit and plant progeny. The corresponding value in the  $X_3$  generation will be  $\pm 30 \cdot 10^{-6}$ . Thus, in those cases in which the analysis is made in the  $X_2$  only one-half of the actual number of mutations are found, the other mutations remaining in heterozygous form. Calculated per ear-progeny the frequencies in the  $X_2$  and  $X_3$  will be about one-third to one-fourth of the corresponding values for the plant progenies. The agreement between the different experimental series is remarkably good.

The influence of metabolism on the mutation process was first investigated by STADLER (1928 b, 1930). He found about a fourfold increase. In the experiments carried out at Svalöf the frequencies do not rise so steeply, but in any case they double or treble (GUSTAFSSON, 1940). On the other hand, the mutation frequencies fall to about one-half if the seeds are desiccated gradually at  $80^\circ$  C. The results obtained from three X-rayed series are given in the following table. More than 300  $X_2$  mutants have been observed in these experiments.

	$80^\circ$	10 % H <sub>2</sub> O	15 % H <sub>2</sub> O	23 hours H <sub>2</sub> O	0,01 % heteroauxin
Golden barley, all mutations	—	10,2	15,4	21,7	26,3
Maja barley, <i>albina</i> .....	—	6,5	—	15,5	—
Ymer barley, <i>albina</i> .....	3,7	8,0	—	—	—
		$\cdot 10^{-6}$			

(All figures calculated per  $r$  unit and plant progeny.)

*Viable chlorophyll mutants.* — Besides chlorophyll mutants that die at seedling stage, there also arise now and again ones that are able to go on living and produce germinable seed year after year. Their seedlings are often pale-green to yellowish-green, and the fully grown plants have a paler tone of colour than what is normally the case. In these mutants earing and ripening are generally considerably delayed. For the most part they belong to the *viridis* group. A few viable *alboviridis* and *tigrina* mutants have been obtained.

In an  $X_3$  generation of Maja barley, where the  $X_1$  seed had been X-rayed with 2.500—25.000  $r$ , there arose eleven *viridis* mutants, one *tigrina*-like *viridis* mutant and one *alboviridis* mutant, all of which

were homozygously viable. The average frequency calculated per  $r$  unit and plant progeny amounts to  $1,3 \cdot 10^{-6}$ , calculated per  $r$  unit and ear progeny it amounts to  $0,3 \cdot 10^{-6}$ . About every twenty-fifth chlorophyll mutant is thus viable in the homozygous state.

*Sterility aberrants.* — Sterile individuals often appear in the progeny of the  $X_1$  plants. The sterility is due partly to structural heterozygosity (chromosomal sterility, according to DOBZHANSKY), partly to recessive factors (deficiencies, genic mutations) that in homozygous form prevent sex-cells from developing or functioning (genic sterility). The two sterility types can be distinguished from each other to a certain extent, the meiosis-disturbing factors often bringing about complete sterility. On the other hand, translocation sterility<sup>3</sup> in barley reduces the fertility by 20—50 %.

A preliminary analysis of the sterility behaviour of the X-rayed Ymer  $b_7$  barley has been performed. The material originates from seed treated with 20.000 and 25.000  $r$ .  $X_2$  progenies were raised from one ear of each  $X_1$  plant. The  $X_2$  generation was classified into (1) progenies consisting of completely fertile or slightly sterile plants, (2) progenies with one or more rather sterile plants (fertility between 75—30 %), (3) progenies with solitary highly sterile plants (fertility about 10 %) and, finally, (4) progenies with solitary completely sterile plants. Group 1 contains unchanged plants as well as plants with small translocations, inversions, deficiencies in heterozygous form. Group 2 contains pronounced structural heterozygotes. Groups 3 and 4 comprise recessive mutations with a changed floral structure, dwarf growth, asynapsis, polymitosis, stickiness and so on as a consequence.

In all, progenies from 558  $X_1$  ears were planted. Of them, 84 gave one or more rather sterile plants (group 2: 15 %), 11 contained one or more very sterile plants (group 3: 2 %), and 17 gave solitary completely sterile plants (group 4: 3 %). The frequency is  $6 \cdot 10^{-6}$  per  $r$  for group 2 and  $3 \cdot 10^{-6}$  for groups 3 and 4 combined. »Meiosis-destroying« mutations thus occur in the  $X_2$  to about as numerous an extent as all chlorophyll mutations taken together. The gross structural re-arrangements that reduce the fertility considerably appear more often.

The segregation in groups 3 and 4 should approach 3 : 1 (the chimaeric structure of the  $X_1$  ears causes a deficiency of recessives), in group 2, on the other hand, more likely 1 : 1 (here, too, with a deficiency for the sterile plants). In group 3 the segregation was 99 : 27, in group 4 it was 188 : 64, i. e. in both cases within the limits of a simple Mendelian segregation. In group 2 the analysis gave 734 completely

or almost completely fertile plants to 319 rather sterile ones. The D/m according to the 3:1 scheme is 4.0 (with a considerable surplus of sterile plants). Hence the sterility in group 2 probably depends to a great extent on translocation heterozygosis.

As sterile and lethal mutants have only been cursorily studied, this analysis cannot be considered as final.

*Vital mutants.* — A most important question from a general point of view is that of the frequency of the vital mutants. In five series

TABLE 5. *Number of vital mutations in three different barley strains.*

Strain	Treatment	Plant progenies		Chlorophyll mutations ( $X_3$ )	Morphological mutations ( $X_2$ )	Physiological mutations ( $X_2$ )
		No. of plants	No. of ears			
Ymer I	10.000 r					
	Changed					
	physiol. ....	802	(2137)	$\pm 12 \%$	11 = 1,4 %	?
Maja I	2.500—25.000 r	1030	(4359)	$\pm 14 \%$	4 = 0,4 %	11 = 1,1 %
Maja II	5.000—10.000 r					
	Changed					
	physiol. ....	1226	(5322)	$\pm 10 \%$	3 = 0,2 %	5 = 0,4 %
		Ear progenies				
Ymer II	10.000—25.000 r	(1208)	1208	$\pm 4 \%$	$\geq 6 = 0,5 \%$	?
Golden I	5.000—10.000 r					
	Changed					
	physiol. ....	(1768)	1768	$\pm 5 \%$	$\geq 2 = 0,1 \%$	$\geq 2 = 0,1 \%$
Golden II	Changed					
	physiol. ....	(862)	862	$\pm 7 \%$	1 = 0,1 %	$\geq 2 = 0,2 \%$

of barley grown 1939—1945 and treated with exactly determined X-ray doses, not only the number of chlorophyll mutants but also the number of morphological vital mutants and to some extent that of the physiological vitals were determined (principally in the  $X_2$  generation). In three series (Ymer b<sub>7</sub> 1, Maja 1 and Maja 2) field progenies were reared from all the ears of the  $X_1$  plants, in three others (Ymer b<sub>7</sub> 2, Golden barley 1 and 2) the different progenies originated from one ear (the principal ear) of each  $X_1$  plant. No physiological variants have been recorded from Ymer barley b<sub>7</sub>, which represents a strain recently produced.

Ymer b<sub>7</sub> gave a high number of morphological mutants in series 1 (10.000 r, different pre-treatment of the original seed). Against  $\pm 100$

chlorophyll mutations in the  $X_2$  there are *at least* eleven morphological mutations, seven of which are erectoid types, two bright-green types, one calcaroid, one *bracteatum*. This means that every tenth mutation of those observed in the  $X_2$  is morphological and vital.

In Ymer 2, where the original seed had been given X-ray doses from (500—)10.000 to 25.000 *r*, 52 cases of chlorophyll mutants were observed against at least six morphological vital types, viz. *erectoides* 17—19 (20), a short-awned erectoid type, one bright-green variant as well as one *intermedium* mutant. Thus, in this series too, every tenth  $X_2$  mutant is morphological and vital.

Maja 1, where the original seed had been given from 2.500 up to 25.000 *r*, shows a lower mutation frequency. Against a calculated number of 154 chlorophyll mutants there are only four morphological and vital types, namely *erectoides* 12—15. Only about every fortieth mutation distinctly observable in the  $X_2$  is thus morphological and vital.

In Maja 2, where the seeds pretreated in different ways had been given 5.000 and 10.000 *r*, there arose 62 *albinas* in the  $X_2$ . This figure corresponds to about 125 chlorophyll mutants. The three morphological and vital mutants consisted of *erectoides* 16 and two bright-green variants. Here too, therefore, every fortieth  $X_2$  mutant deviates morphologically and is vital.

Golden barley 1 (cf. GUSTAFSSON, 1940, the 1939 series: 17—24) produced in the  $X_2$  generation 92 chlorophyll mutants and two (to three) vital morphological mutants (*erectoides* 9 and 10, possibly also a *tetrastichum* variant). About every fiftieth mutant is thus vital and morphological.

The series named Golden barley 2 in Table 5 originates from seeds which had been treated in different ways before being X-rayed. The dose was not measured in *r* units. A single vital morphological type (*erectoides* 11) was observed. In addition, 63 chlorophyll mutants were formed. Hence, in this series every sixtieth  $X_2$  mutant is vital and simultaneously morphologically changed.

The three barley strains thus give vital morphological mutants in the proportion of 1 in 10, 1 in 40, 1 in 52, the average being 1 in 23. Ymer  $b_7$  seems most liable to produce morphological mutants, Golden barley least liable, Maja having about a mid-position. The different capacity of the three strains to produce morphological mutants corresponds to different mutation frequencies in spontaneous material. As was earlier remarked, Golden barley represents a very old pure line

(isolated before 1900), while Maja and Ymer are recent cross-products with Golden barley as one of the original types. Ymer barley is the most complex as well as the last formed product.

Several physiological mutants have been isolated in the  $X_2$  and  $X_3$  of the Maja and Golden-barley series. They have often been observed already in the  $X_2$ , but the range of modification of normal material is so wide that their nature of mutations cannot always be established until in the  $X_3$ . Individual progenies in great numbers should preferably be raised in the  $X_3$  and  $X_4$  and then searchingly compared with the maternal line. This is however possible only to a limited extent. The mutants observed are therefore only a fraction of those that have actually been formed. Unless special precautionary measures are taken (p. 51), spontaneous crossings and solitary seed intermixtures may sometimes be classed as mutants. Physiological variants cannot be awarded the same general value as evidence as morphological ones. In the six series which have been most thoroughly examined, and in which the sources of error have been successively eliminated, at least twenty cases of completely fertile physiological mutations have been observed (Table 5).

In Maja 1 eleven physiological mutants correspond to about 154 chlorophyll mutants, i.e. a proportion of 1:14. In Maja 2 five physiological mutants arose. The proportion in this case ( $\pm 125$  chlorophyll mutants) is 1:25. In Golden barley 1 two physiological mutants were isolated as against 92 chlorophyll aberrants. This means a proportion of 1:46. Finally, in Golden barley 2, two or probably three physiological types were observed as against 63 chlorophyll mutants, i.e. a ratio of 1:32.

The Maja barley seems more liable to give physiological mutants than the Golden barley, *which as a rule behaves very stably*. On an average the four series gave about two vital physiological mutants as against one morphological.

Of the morphological mutants enumerated here, eleven have up to now been tested in yield trials (p. 60). Four of them have given about the same yield as the mother-line, one has shown a better result.

All the fertile mutants produced in the physiological group have been tested in respect of their yield. Of the twenty types, nine are equal to and two probably superior to the maternal lines, i.e. show about the same ratio as within the morphological group.

## V. THE MODE OF ORIGIN OF INDUCED MUTANTS.

### 1. SOURCES OF ERROR AND METHODS OF CONTROL.

The control of the mutants and their mode of origin have been sharpened as the investigations have advanced. However, even in the predominantly self-fertilizing varieties of cereals — barley, oats and wheat — spontaneous crossing occurs to a varying extent in different lines (STEVENSON, 1928). The risk of crossings can nevertheless be eliminated by means of suitable experimental conditions. Another source of error consists in contamination of seed material or admixture of foreign seed. An admixture is however rather easy to discover, as in such cases the progenies from all the ears deviate in a similar manner from the control.

The following precautions have been successively adopted. (1) The seed material to be X-rayed is taken from élite material of a pure line, often from individual plants, which also have to supply control series. If a non-homogeneous commercial strain is to be X-rayed, only morphological aberrants are isolated. (2) The  $X_1$  (as well as the  $X_2$ ) generation should as far as possible be grown apart from other field material of the same variety of plant. (3) Each  $X_1$  plant is harvested and threshed separately and its kernels are sown separately ear for ear. (4) The variants that arise are scrupulously compared with other lines and strains in the assortment. (5) In specially important model experiments use is made of lines that are recessive for certain easily observable properties. For instance, seed of Pudel wheat functioned in the first X-raying experiments in wheat. This line is homozygous for white kernel colour and glume hairiness. Other lines having these properties are not in culture. The kernel colour of the observed variants show whether crossings or mutations have occurred. (6) An extensive control material is sown.

In two-rowed barley the individual control has been carried so far that every mutant arising can be referred not only to a definite  $X_1$  plant but also to a definite  $X_1$  ear, in which the number of well-developed kernels, as well as the number of empty flowers, is exactly known. In six-rowed barley, wheat and oats it takes up far too much time to determine the exact sterility ear for ear. However, even these kinds of plants have been subjected to as exacting control as possible, especially by way of extensive cultivation of progenies from non-irradiated plants.

It may be added that so careful a testing as this is hardly necessary

if only morphological variants are to be produced. As a matter of fact, so far as these are concerned it is easy to determine whether they originate from mutation, spontaneous crossing or admixture. On the other hand, physiological variants must be scrupulously followed and controlled, since many of them differ only slightly from the maternal line.

As previously mentioned, admixtures can be readily unmasked by the fact that all the ear progenies then deviate. *Individual* ear progenies, on the other hand, may be homozygous for a recessive mutation already in the  $X_2$ . Some examples may illustrate this. The kernels from three ears of an  $X_1$  plant out of Ymer b<sub>7</sub> were sown. Seven of nine kernels from ear 2 germinated and all were »*albina*». The two remaining ear progenies were normally green. In another case an  $X_1$  ear with eight kernels germinated. The six seedlings that arose represented the rare mutation *alboxantha*. In a third case three ear progenies from an  $X_1$  plant were sown. From one of the ears there arose eight identical seedlings of a yellow tone of colour (chlorophyll mutation) and homozygously viable, although greatly delayed in their earing. At times ears that are homozygous for a recessive character (e. g. *albina* or *xantha*) can already be observed on the  $X_1$  plant.

Some examples will illustrate the genesis of the mutations:

*Erectoides* 12 arose out of Maja barley. From 865 X-rayed seeds (2.500 r) 557  $X_1$  plants were obtained with an average fertility of 89 %. The  $X_1$  plant in question showed 95 % fertility, the mutated ear 91 %. The plant possessed nine ears. In the progeny from ear 4 two erectoid plants were observed. Of five selected  $X_2$  plants three were normal in the  $X_3$ , one segregated for *erectoides*, one was a constant *erectoides*.

*Erectoides* 12 deviates not only from Maja barley but also distinctly from all barley lines under cultivation. The  $X_1$  generation in question was kept separate. The extensive  $X_2$  generation was sown later than the other barley material and apart from this.

*Erectoides* 16 likewise arose out of Maja. The seed material was soaked in water for 21 hours prior to being X-rayed (10.000 r). From 1436 sown seeds only 46  $X_1$  plants were harvested, with an average fertility of 66 %. The  $X_1$  plant concerned gave twelve ears and possessed 71 % fertility. Fifteen kernels in ear 6 (65 % fertility) gave an  $X_2$  generation consisting of seven plants. One of these was recorded as »early ripening». New progenies were raised from five of these seven  $X_2$  plants. Three plants were normal, one heterozygous and one homozygous for *erectoides* 16. Seed material from the two remaining  $X_2$  plants was sown in the spring of 1945. One was heterozygous for the mutation in question, one did not differ from the mother-line.

The same  $X_1$  plant and the same  $X_1$  ear gave rise to a physiological mutant (44/109) that deviates from the maternal line, principally by broader leaves. *Erectoides* 16 differs in shape of ear as well as by its extreme earliness. Neither

variant can have arisen as a consequence of spontaneous crossing or seed admixture. Besides them, a *lutescens* mutant was also formed in the  $X_2$  progeny from ear 11. The  $X_2$  plants from ear 5 were completely sterile.

The cultivating conditions for the  $X_1$  and  $X_2$  generations were the same as for *erectoides* 12.

An example of a fully controlled still more complex behaviour is offered by a Golden barley series that was already X-rayed in 1937 (1937: 110, GUSTAFSSON, 1940). The treatment was so intensive that from 508 sown seeds there arose only thirty  $X_1$  plants, with an average fertility of 32 %. The  $X_1$  plant in question possessed a fertility of 37 %. Out of it there arose, *inter alia*, a very broad-leafed »winter-barley like» mutant (44/65, cf. p. 31, difference for leaf-breadth  $3,6 \pm 0,4$  mm.). The same ear progeny gave a physiological variant that differed from Golden barley by somewhat broader leaves (44/23, p. 33, difference for leaf-breadth  $1,6 \pm 0,2$  mm.). In another ear progeny there appeared an *albina* mutant and at the same time a straw-stiff mutant (42/13). The winter-barley like mutant appeared in two of the eleven ears. (There are three to four ear initials in one barley embryo: hence, several of the ears that arose on the harvested plants were secondary and could therefore give the same mutant.)

## 2. $X_1$ FERTILITY AND TYPE OF MUTATION.

As the fertility of the maternal plant is known for most of the induced mutants, it is possible to determine the X-ray effect required to induce a certain mutation or mutation type. To take an example from Ymer b<sub>7</sub> barley: The mutant »short-awned» arose from an  $X_1$  plant with only 42 % fertility, *erectoides* 22 from an  $X_1$  plant with 92 % fertility. This decrease in fertility illustrates the nuclear changes required to enable the two mutants to arise (if, of course, these fertility figures are representative). »Short-awned» thus requires for its origin a greater change in the genotypic constitution of the  $X_1$  seed in question than *erectoides* 22.

Another example may be appended to this: GUSTAFSSON (1940) considered that he was able to determine that *viridis* mutations preferably arise in the progeny of sterile  $X_1$  plants while the *albina* mutations are readily formed in the progeny of fully fertile plants also. The former would thus occur predominantly in fertility classes in which translocations, inversions, etc. have accumulated. (This view has been criticized by MÜNTZING, 1942, as well as by FREISLEBEN and LEIN, 1943; see GUSTAFSSON, 1946 b.) In the mutation experiments of the last five years 471 *albina* mutants have been produced. The corresponding  $X_1$  plants possessed an average fertility of  $77,0 \pm 0,7$  %. The *viridis* mutants, numbering 136, showed an average  $X_1$  fertility of  $71,4 \pm 1,4$  %. The difference is statistically significant. Hence, for their origin the

*viridis* mutants need between 5 and 6 % lower average fertility in the  $X_1$  plants than the *albina* types.

The following series is obtained:

Mutation type	<i>albina</i>	<i>xantha</i>	<i>alboviridis</i>
Average $X_1$ -fertility: . . . . .	77,02 $\pm$ 0,68 %	74,09 $\pm$ 1,38 %	74,07 $\pm$ 1,47 %
Number of mutants: . . . . .	471	66	108
	<i>viridis</i>	physiological mutants	morphological mutants
	71,40 $\pm$ 1,43 %	71,03 $\pm$ 3,72 %	67,50 $\pm$ 3,34 %
	136	29	36

According to these data chlorophyll mutants, especially *albinas*, are on an average considerably more liable to arise than physiological and morphological mutants (i.e. result from less drastic changes). The differences however are not significant, except in the case of *albina* and *viridis* types ( $D/m = 3,6$ ), as well as probably in that of *albina* and morphological mutants ( $D/m = 2,8$ ). Physiological mutants (most closely comparable with »Kleinmutationen» in BAUR's sense) arise, regarded as a group, more frequently than morphological, although many of them are discovered only after careful  $X_3$  analysis, and they also seem to be formed somewhat more readily.

Of far greater interest, however, is the fact that the morphological mutants can be divided into groups that possess distinct sterility properties. One group comprises the bright-green, »non-glaucous» mutants, which have been described on page 29. Another group consists of the erectoid mutants, which are sharply delimited and easy to define (p. 20). To a third group belong the rare mutations which more or less drastically change the phenotype and in several cases cause the spikelets and flowers to get quite a new structure.

The degree of pleiotropism runs roughly parallel with the increased degree of drasticity. Certainly the bright-green mutants deviate sharply from the maternal lines, though only in individual characters. Many of the erectoid types are very pleiotropic (p. 23). This condition is probably due to the fact that gene changes and structural rearrangements have simultaneously occurred. Reasons arguing in favour of this assumption have been submitted earlier. Among the drastic mutants are ranked *calcaroides*, *bracteatum*, *densinodosum* (2 cases), »lemma-like glumes» (2 cases), *intermedium*, *tetrastichum*, short-awned, »winter-barley like» mutants with altered flower structure (2

cases). The spontaneous mutant *scirpoides* ought also to be placed in this group. These three groups show significant differences in plant as well as ear fertility in the  $X_1$  generation, as the following table shows (GUSTAFSSON, 1946 a).

	»Non-glaucous»	<i>Erectoides</i>	Drastic mutants
(A) Average fertility of the mutated $X_1$ -plants . . . . .	78,50 $\pm$ 6,55 %	73,00 $\pm$ 2,71 %	51,27 $\pm$ 6,07 %
(B) Average fertility of the mutated $X_1$ -ears . . . . .	92,75 $\pm$ 3,92 %	74,57 $\pm$ 4,39 %	55,82 $\pm$ 7,90 %
Number of mutants . . . . .	4	21	11

In group (A) the difference between erectoid and drastic mutants is significant ( $0,01 > P > 0,001$ ), as it also is between the bright-green and the drastic mutants ( $0,01 > P > 0,001$ ).

In group (B) two differences are significant, between »non-glaucous» and erectoid mutants on one hand ( $0,01 > P > 0,001$ ) and »non-glaucous» and drastic mutants on the other ( $P = 0,001$ ). The difference between erectoid and drastic mutants corresponds to a P value of 0,05.

The fact that the three groups are significantly separated from one another is also shown by an analysis of variance. The quotient inter/intra-class gives a P value which in both (A) and (B) lies between 0,01 and 0,001.

These results may be construed in two ways: *either* the individual genes which, after mutating, drastically alter the phenotype are so stable that they are affected only by the very strongest X-ray action, *or* the drastic morphological changes require, in order to be realized at all, a complete reconstruction of the chromosome material, for instance changes in one principal gene accompanied by drastic re-arrangements in the chromosomes, in modifiers, polygenes and so on.

The yield of the morphological mutants falls in the measure the changes become greater. Three tested bright-green mutants gave an average yield index of 101 (against 100 for the mother-lines). Fourteen erectoid types gave the relative value 88. Of the drastic mutants, only two have been tested so far (»lemma-like glumes» 1 and 2). These gave a yield index of 84. Of the rest, the *densinodosi* are completely sterile. *Calcaroides* is vegetatively distinctly weaker than the maternal line. In *bracteatum* the ears bend just before ripening and hang down, a clearly negative character. The two »winter-barley like mutants» give a low yield of poorly ripened kernels when grown as spring barley; grown as winter barley they are unable to endure the winter. *Scirpoides*, the only spontaneous drastic mutant, is distinctly

weakened, with smaller ears and fewer kernels. Hence the drastic mutants — at any rate in the area of cultivation of the original line itself — give a substantially lower yield and are in some cases non-vital.

An interesting fact is that the yield within the erectoid group also falls in the measure the deviations from the original line are accentuated. In the following table the yield, straw-length and ear-length of the maternal lines have been put at 100.

Yield	Slightly changed ear- and straw-length; kernel character unchanged	Greatly reduced ear-length (rel. fig. < 80)	Greatly changed straw-length (rel. fig. < 90 or > 110)	Greatly changed ear- and straw-length	Changed kernel characters
Golden barley mutants .....	101, 97, 91	92, 91	83	—	89, 83
Maja barley mutants .....	95	97	100	76, 51 <sup>1</sup>	—

### 3. EXPERIMENTAL CONTROL OF THE MUTATION PROCESS.

The important problem of whether the mutation process can be experimentally controlled has in recent years been made the subject of special investigations. The results are as yet meagre but seem to go in a positive direction. RHOADES's important investigations of 1938 and 1945 pointed clearly in this direction. Before that, DEMEREC (1937) had observed that a definite gene in the second chromosome of *Drosophila melanogaster* was able to increase the mutability of the whole genome very considerably. In RHOADES's case in maize the labilizing effect proved to be specific. The mutability was increased solely in a definite gene. The gene  $a_1$  is very stable in the presence of gene  $dt$  and instable in company with the gene  $Dt$ . Other genes modify the effect of  $Dt$ . Chromosome aberrations do not occur.

In 1940 GERSHENSON observed that *Drosophila* larvae fed with the sodium salt of thymonucleic acid — whether followed by X-raying or not — give predominantly mutants that affect the structure of the wings. This result has been contended by RAPOPORT (1940). The

<sup>1</sup> *Erectoides* 14 with the relative figure 76 has also changed kernel character, *erectoides* 15 with the figure 51 has uncoloured nerves on the lemmas.

same year GUSTAFSSON considered he was able to show that the so-called *alboxantha* mutation in barley (see below) arises in considerably higher frequency if the seeds are soaked in water or heteroauxin before being X-rayed. The next year (1941) DOTTERWEICH and SCHMIDTKE believed they were able to prove that, if *D. melanogaster* is fed with follicle hormone before irradiation, the visible mutants increase in number and change character as well. Mutations that alter the type of the wing are replaced by such as effect the bristles and the venation of the wings. With very high quantities of hormone the stability of the genome is considerably reduced. The visible mutants increase many-foldly as compared with the spontaneous cultures. Bristle mutants dominate. Especially the sex-linked gene *scute* and the autosomal gene *Stubble* mutate. Genes having definite phenotypic effect but located in different chromosomes are thus labilized by a change in the external environment.

The *alboxantha* mutation in barley is very rare both as a spontaneous and an induced product. Probably it is due to changes in a definite gene (this is not yet proved). The material collected since 1940 corroborates earlier observations, viz. that *alboxantha* mutants are considerably more liable to arise from seeds soaked in water and auxin than from normally and artificially dried seed material.

In laboratory experiments (the only reliable method) with Golden barley and Maja barley eleven *alboxantha* mutants arose, which were distributed over different seed materials as follows:

	Dry seeds contain previous to irradiation		Dry seeds previous to irradiation pre- treated with	
	10 %	15 %	H <sub>2</sub> O	Auxin
	H <sub>2</sub> O	H <sub>2</sub> O	23 hours	23 hours
Number of X <sub>1</sub> -progenies . . . . .	2184	586 = 2770	501	702 = 1203
» » chlorophyll mutants . . . . .	254	66 = 320	109	128 = 237
» » <i>alboxanthas</i> . . . . .	0	1 = 1	3—4	6 = 9—10
» » <i>tigrinas</i> . . . . .	7	3 = 10	1	0 = 1

The distribution dry *contra* soaked series gives  $P = 0,001$  for *alboxantha* as against non-*alboxantha* mutants (according to YATE's correction  $0,01 > P > 0,001$ ), while for *alboxantha*-mutated as against non-*alboxantha*-mutated X<sub>1</sub> plants it gives  $P < 0,001$  (before as well as after correction for low number of mutants).

In the progenies from Ymer b<sub>7</sub> (chiefly dry seed with 12 % H<sub>2</sub>O

which had received 500—25.000 r) not a single *alboxantha* mutant arose in the indoor material. On the other hand, in the field experiments, which do not allow of a fully reliable classification, possibly one *alboxantha* mutant was formed (after 25.000 r). Dry seed from Maja barley did not yield any *alboxantha* even after the highest doses (25.000 r).

This indicates that the cell-nuclei must be in a certain physiological state for *alboxantha* mutations to arise. If the high hydration is not present, even the highest X-ray doses are unable to induce them.

Another mutation type, *tigrina*, likewise very rare, shows in the same material a contrary state of things (see above). The difference as against *alboxantha* is statistically significant ( $P < 0.001$ , with or without YATE's correction). It has arisen five times from Ymer b<sub>7</sub>, in all cases in dry series.

The identity of the various *alboxantha* mutants has admittedly not yet been cleared up, but even if several genes should be responsible for one and the same phenotypical effect, most of them must respond in quite a definite manner to changes in the state of the cells.

Another example may be submitted to show that mutations do not arise quite at random. *Xantha* mutants, as they are defined and classified by me, show a tendency to accumulate in fertility regions having a slightly but distinctly reduced fertility (70—90 %) and to avoid regions with full fertility (90—100 %) or low fertility (0—70 %) (GUSTAFSSON, 1940, 1946 b). On an average they show no significant average decrease in the fertility of the X<sub>1</sub> plants as compared with the *albina* types (see the table on p. 54), but the distributions are entirely different:

Fertility of the X <sub>1</sub> plants										
	0—10	— 20	— 30	— 40	— 50	— 60	— 70	— 80	— 90	— 100 %
<i>Albina</i> .....	1	2	5	18	36	66	115	133	95	= 471
<i>Xantha</i> .....				2	3	13	20	25	3	= 66

The difference in distribution is statistically significant (in a  $2 \times 2$ -table  $P$  will be greater than 0,001 but less than 0,01, in a  $2 \times 5$ -table  $P$  is 0,01).

An analysis of the distribution of *albina* and *xantha* mutants in relation to the number of X<sub>1</sub> plants in the different fertility classes shows that the *albina* types are regularly distributed over the whole of the area, while the *xantha* types accumulate in regions with a reduced fertility. Two of the three *xantha* mutants, the mother-plants

of which showed 90—100 % fertility, arose from plants with 91 % fertility, i.e. values below those of the control plants (96—97 %). The mother-plant of the third *xantha* mutant certainly possessed 96 % fertility, but in the same ear progeny there also arose one *albina* and one *alboviridis* mutant. In spite of the apparently normal fertility, therefore, a very strong X-ray effect must have occurred.

There is hardly any doubt that the *xantha* mutations — whatever their innermost nature may be — generally require a more powerful X-ray effect than the *albina* mutations; they cannot arise in the progeny of completely fertile  $X_1$  plants. To obtain *xanthas* it is necessary to induce sterility and raise progenies from distinctly but weakly sterile plants.

Judging from these results, therefore, the mutation process does not proceed at random; it is pre-determined, can be controlled by altering the *cell environment* in a definite manner. RHOADES's exceedingly important investigation, already cited earlier in this work, shows that individual genes are stabilized or labilized when the gene environment is changed.

Experiments are in progress on a large scale to endeavour to direct the mutation process methodically so far as vital mutants are concerned. As was mentioned earlier (p. 55), the three groups of morphological mutants show quite different sterility dependence.

## VI. YIELD AND QUALITY.

### 1. THE YIELD OF THE INDUCED MUTANTS.

#### A. SVALÖF TRIALS.

The yield of the barley mutants has been determined by trials spread over several years at the barley department of the Swedish Seed Association. During the first three years these trials were being conducted (1940—1942) this department was under the direction of Prof. H. NILSSON-EHLE, subsequently (1943—1945) under that of Dr. I. GRANHALL. The department's assistants Mr. C. G. VON SYDOW and Mr. C. BERGSTRÖM were responsible for the practical parts of trials during the same period.

The yield trials were laid down in the following way:

1940 — Preliminary series, see GUSTAFSSON, 1941 b, p. 344.

1941 — Two series. A. Machine-sown trials, plots  $7 \times 1.36$  m. ( $9.52$  m<sup>2</sup>), four replications, 200, 300, 400 and 200 kg of saltpetre per hectare. B. Hand-sown trials (= 1940), four replications, same manuring as in A.

1942 — A. Machine-sown trials, plots  $6,5 \times 1,86$  m. ( $8,84$  m<sup>2</sup>), four replications, manuring as in 1941. B. Hand-sown trials ( $\approx$  1940) but only three replications: 200, 300, 400 kg saltpetre.

1943 — A. Machine-sown trials, plots  $6,5 \times 1,86$  m. ( $8,84$  m<sup>2</sup>), four replications, manuring as in 1941. B. Hand-sown trials ( $\approx$  1940), three replications.

1944 — A. Machine-sown trials, plots  $6 \times 1,58$  m. ( $9,48$  m<sup>2</sup>). Three replications. Manuring 200, 300 and 400 kg saltpetre per hectare. B. Small machine-sown trial, plots  $4 \times 0,75$  m. ( $3$  m<sup>2</sup>), three replications: 200, 300, 400 kg saltpetre.

1945 — A. Machine-sown trials, plots  $7 \times 1,28$  m. ( $8,96$  m<sup>2</sup>), three replications, manuring 200, 300, 500 kg saltpetre. Mutants out of Golden barley. B. Same as A. Mutants out of Maja barley. C. Machine-sown trial, plots  $3,7 \times 1,28$  m. ( $4,74$  m<sup>2</sup>), two replicates, 200 kg nitrate of lime. Mutants out of Golden barley. D. Same as C. Mutants out of Maja barley. E. Same as C. Mutants out of Maja barley. F. As for C but only one plot of each number. Mutants from Maja and Ymer barley.

Mutants showing reduced fertility were not included in the yield trials, nor were mutants that are fertile but vegetatively weak or abnormal (dwarfs, too late variants, etc.). A strong selection for rich tillering, for general viability and for fertility had already been carried out in the segregating  $X_2$  and  $X_3$  generations.

The sowing and harvesting were effected by the barley department and carried out by the standard methods applied by the Swedish Seed Association.

In the years 1943—1945 yield trials were also laid down at some of the branch stations of the Seed Association.

The mutual relation of the standards (the mother-lines) as well as their absolute yield has some significance in this connexion. In six years of mutation experiments Maja barley has been on an average 12 % over Golden barley (lowest 7 % in 1943, highest 21 % in 1942). Maja barley averaged 4740 kg per hectare in the machine-sown trials of 1941—1945. The official trials for Malmöhus län, the administrative district in which Svalöf is situated, show for the six-year period 1938—1943 a superiority by 10 % for Maja barley and for the same strain an absolute yield of 4140 kg per hectare. There is thus rather good agreement between the Svalöf figures and the official ones. Mutants out of Ymer  $b_7$  were only tested one year (1945). Ymer barley  $b_7$  itself gave the relative value of 97 in comparison with Maja barley. In the Seed Association's yield report for Ymer barley (strain  $b_1$ ; GRANHALL, 1944) a relative figure of 99 is given.

#### a. Morphological mutants.

In the course of years nineteen vital morphological mutants have been tested for their yield. As shown by the following survey, the mutants have on an average produced yields 90 % of that of the maternal lines. Seven mutants stand about level with the mother-line or higher.

These are naturally the only ones of any great interest in this connexion.

		Yield (mother-line = 100)						Average
		45—55	65—75	85—95	105—115			
Golden mutants	.....		3	5	2	= 10	89,5	
Maja mutants	..... 1		1	1	4	= 7	88,0	
Ymer mutants	.....			1		1 = 2	(98,0)	
Total	..... 1		4	7	6	1 = 19	90,0 ± 3,0	

Respecting *erectoides* 1 (Table 6) there are now so many data as to warrant the definite assertion that it is as highly yielding as the mother-line. It is further distinctly straw-stiffer (for the three years 1943—1945 graded at 7,4 against 5,7 for the mother-line). The 1000-grain weight and hectolitre weight are about the same as in the mother-line, and so are the protein and starch contents. The ripening time is slightly longer (one or two days).

*Erectoides* 7 is a little inferior in respect of yield. Its straw-strength is however higher (the same as in the preceding case). It has a somewhat lower 1000-grain weight and hectolitre weight, and ripens distinctly later (two or three days).

*Erectoides* 12 out of Maja barley has naturally enough a considerably higher yield than the two preceding mutants and is fully on a level with the mother-line. Its straw-strength is better (8,8 against 8,0), its 1000-grain weight and hectolitre weight about the same, likewise its earliness (possibly one day later).

*Erectoides* 16, which showed a poor result in the trials of 1944 but a considerably better one in those of 1945, is the most interesting of the morphological mutants from a practical point of view, especially on account of its pronounced pleiotropism. Its yield lies a little under that of the mother-line, but this is undoubtedly due to its extreme earliness (six or seven days). Its 1000-grain weight is distinctly higher (41,9 against 38,5 grams), and so is its straw-strength (8,8 against 7,9). In 1945 it was also tested in northern Sweden, where its earliness may have some value. It has been crossed on a large scale with the earliest commercial strains.

*Erectoides* 13 is especially interesting on account of its extreme straw-strength (9,9 against 7,5). The hectolitre weight is higher but the 1000-grain weight somewhat lower than the mother-line's. It yields somewhat less.

The two bright-green mutants, which judging from the figures

rank level with or above the mother-line in yield, have not as yet been tested sufficiently long for a reliable opinion to be given. Mutant No. 3 was included in a small trial. Two different pedigree lines, however, were tested and gave the relative values 110,0 and 101,7. In other qualities the two mutants stand close to the mother-lines.

#### b. Physiological mutants.

Thirty-one vital mutants have been tested in yield trials in the course of years, fifteen of them originating from Golden barley, six-

TABLE 6. *The yield of the seven best morphological mutants in barley.*  
(Parents = 100.)

V a r i e t y	1940	1941	1942	1943	1944	1945	Average
Golden barley .....	100	100	100	100	100	100 100	100
<i>Erectoides</i> 1 .....	101,3A	100,2A	101,3A	93,6A	102,5A	102,3A 104,9C	100,9
<i>Erectoides</i> 7 .....			92,9A	96,7A	100,4A	97,7A 100,0C	97,5
Maja barley .....					100	100 100	100
<i>Erectoides</i> 12 .....					99,5A	101,3B 99,2D	100,0
<i>Erectoides</i> 13 .....					93,2A	101,0B	97,1
<i>Erectoides</i> 16 .....					85,9A	100,5B 99,5E	95,3
Bright green 2 .....					95,8B	108,8B	102,3
Ymer barley b <sub>7</sub> .....						100	100
Bright green 3: ped. 1						110,0F	105,9
ped. 2						101,7F	

(The letters behind the yield figures denote the experiment series of the year in question. Cf. p. 60.)

teen from Maja barley. The average yield amounts to 94 %, i.e. a little higher than for the morphological group. The difference is not significant, however.

	Yield (mother-line = 100)						M
	65	— 75	— 85	— 95	— 105	— 115	
Golden mutants .....	1	1	7	5	1	= 15	93,0
Maja mutants .....			3	4	7	2	= 16 94,9
Total .....	1	4	11	12	3	= 31	93,9 ± 1,4

Fifteen mutants are about equal to or better than their mother-lines (Table 7). Of the three mutants with relative values between 105 and 115, one (44/7) was tested in three annual yield trials, and

TABLE 7. *The yield of the fifteen best physiological mutants in barley.*  
(Parents = 100.)

Variety	Characteristics	1940	1941	1942	1943	1944	1945	Average
<i>Golden barley</i>		100	100	100	100	100	100	100
44/2	Late, tall. ....	108,2A	110,0A	88,7A	99,2A	109,3A	101,8A	102,9
44/9	Broad-leafed, late ...				100,5A	104,7A	99,5A	101,6
44/7	Large seeds .....				101,6A	111,3A	113,7A	108,9
44/23	Broad-leafed .....			100,7B	98,0B	101,9B	103,3A	101,0
44/14	Straw-stiff, early							
	ped. 1 .....				105,1B	103,4A	103,6A	
	ped. 2 .....				110,3B	97,8A	102,5A	103,8
44/25	Tall, stiff							
	ped. 1 .....				101,7B	97,6B		
	ped. 2 .....				95,1B	98,7B		98,3
<i>Maja barley</i>						100	100	100
44/18	Straw-stiff, early .....					94,1A	106,5B	100,3
44/30	Seeds differently coloured .....					103,0B	102,8B	102,9
44/31	Broad-leafed, late ...					100,2B	98,0B	99,1
44/88	Early .....						98,6D	98,6
44/76	Waxy							
	ped. 1 .....						102,8D	
	ped. 2 .....						114,1F	108,5
44/85	Tall, long ears							
	ped. 1 .....						93,8D	
	ped. 2 .....						96,3D	95,1
44/89	Short							
	ped. 1 .....						114,3D	
	ped. 2 .....						109,5D	109,6
	ped. 3 .....						105,1D	
44/91	Tall, seeds differently coloured							
	ped. 1 .....						106,7D	
	ped. 2 .....						99,7F	103,2
44/100	Short							
	ped. 1 .....						98,7E	
	ped. 2 .....						94,9E	96,2
	ped. 3 .....						94,9E	

hence the mean value of these should be rather reliable. It deviates especially by its large kernels (the 1000-grain weight for the years 1943—1945. was 43,2 as against 39,7 for the mother-line), its broad

leaves, short straw and at the same time higher straw-strength (6,<sub>3</sub> against 5,<sub>3</sub>), and is somewhat earlier than the maternal line (one day).

44/89 is an apparently high-yielding mutant out of Maja barley, which it much resembles. It has a shorter culm, somewhat inferior straw-strength but as against this it is about three days earlier in ripening. Three different pedigree lines of this mutant were tested in 1945, each in two replications. All were substantially above Maja in yield.

44/76 has a deviating kernel colour and is more waxy than Maja. It was tested in two series in 1945. The relative value is not so reliable as in the preceding case. Its 1000-grain weight stands somewhat higher. The ripening time has been shortened a little (one day).

Of the remaining twelve special mention may be made of the following.

Much like Golden barley are 44/2 and 44/23. The former, a »late, tall» mutant, has been tested for six years and given the relative value of 103. In 1945 it was ten centimetres taller than the mother-line and five days later in ripening. Its straw-strength seems to be good in spite of the high culm (1943—1945: 6,<sub>2</sub> against 5,<sub>3</sub> for the mother-line). It is characterized by very great fluctuations in yield. These are probably connected with the variation in precipitation during the vegetation period. Following the relatively dry summers of 1940, 1941 and 1944 the yield was at 108, 110 and 109. During 1942, 1943, 1945 with their rich rainfall it was at 89, 99 and 102 (see below). In the year 1942 there was a heavy rainfall just at the time the mutant was earing (fertilizing time), with much lodging as a result.

	1940	1941	1944	1943	1945	1942
Precipitation from sowing						
to harvest, mm. . . . .	135	173	206	225	256	265
Relative yield . . . . .	108, <sub>2</sub>	110, <sub>0</sub>	109, <sub>3</sub>	99, <sub>2</sub>	101, <sub>8</sub>	88, <sub>7</sub>

44/23, a segregate out of the same X<sub>1</sub> ear as gave the »winter-barley like» mutant 2, deviates from Golden barley by its somewhat broader leaves (p. 32). Its 1000-grain weight is likewise a little higher (43,<sub>2</sub> against 38,<sub>5</sub> grams). It seems to be one or two days later in ripening.

Compared with the mother-lines, two mutants are distinctly straw-stiffer, 44/14 (8,<sub>1</sub> against 6,<sub>2</sub>) and 44/30 (8,<sub>9</sub> against 7,<sub>2</sub>). Three weaker in straw are 44/25, 44/88 and 44/91. Substantially earlier (up to four days) are 44/18, 44/85 and 44/88. The last-mentioned is also characterized by high straw, long ears and high 1000-grain weight (44,<sub>1</sub>

against 37,0). Other valuable mutants listed in Table 6 fluctuate round the mother-lines in earliness, straw-strength, 1000-grain weight, straw-height, ear-length.

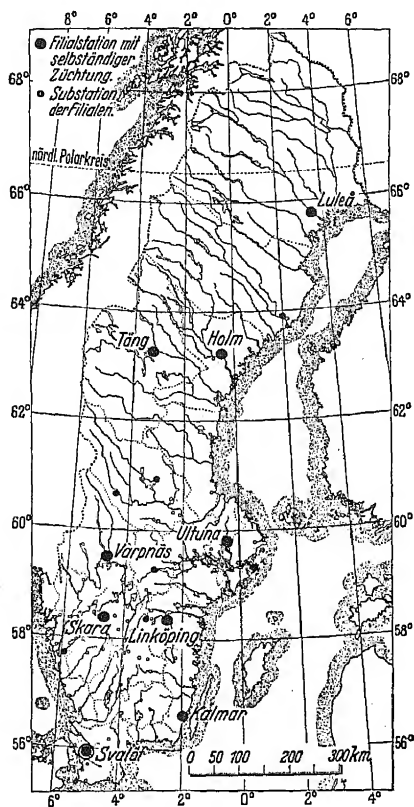
In addition to these thirty-one mutants, some variants have been included in one or more annual yield trials. As it cannot be definitely proved that they have arisen as induced mutations, they are not included in the survey on page 62. One of them, 44/3, an extremely waxy, bushy and peculiar variant, originated from Golden barley, was discussed on p. 33. It deviates considerably from all barley lines at Svalöf. Besides the above-mentioned two qualities it is characterized by extreme straw-strength (9,3 against 6,2). Unfortunately, it possesses a low 1000-grain weight (33,7 against 39,7), to a large extent owing to the awns being shed at an early stage. The yield was:

1942	1943	1944	1945	Average
108,8	94,8	100,2	104,6	102,1

#### B. TRIALS AT THE BRANCH STATIONS.

The system of branch stations of the Swedish Seed Association takes in the whole country (see Map 1). In recent years certain mutants have been tested in regular trials at the Kalmar station, Ultuna station, Västernorrland station (Län-näs, close to Holm), and the Luleå station. The results are only to be regarded as preliminary. The trials will be repeated and extended.

*Kalmar station.* — In the year 1945 the trials were stationed at the Ekerum farm in the island of Öland opposite the town of Kalmar. The district is characterized by severe summer drought and a dry, calciferous, rather unproductive soil. Five Golden barley mutants were tested. The plots were 20 m<sup>2</sup> in size with four replicates of each number. For Golden barley the crop per hectare amounted to 3025 kg. A land variety peculiar to Öland (Löt) gave 2965 kg. The figures were:



Map 1. The stations of the Swedish Seed Association.

	Yield	1000-grain weight	Ripening time, days
Golden barley .....	100	36,7	102
<i>Erectoides</i> 1 .....	106,8	36,5	102
44/2 .....	101,5	36,0	103
44/3 .....	98,5	33,9	100
44/7 .....	120,1	40,4	100
44/14 .....	109,9	36,9	100
Land barley .....	98,0	38,9	101

The high yield of *erectoides* 1, the large-kernelled mutant (44/7) and the upright early mutant (44/14) may be specially noted. The 1000-grain weight for 44/7 is very high, whereas it is low for 44/3.

*Ulluna station.* — This branch is distinguished by severe drought in early summer. Four mutants out of Golden barley were compared in 1944 and 1945 with their mother-line, likewise four mutants out of Maja barley in 1945. The size of the plot was 7,5 m<sup>2</sup> the first year, 10 m<sup>2</sup> the second year, and the number of replicates was four. For Golden barley the hectare yield in 1944 amounted to 2925 kg (manuring 200 kg superphosphate per hectare) and in 1945 to 3805 kg (manuring as in 1944); for Maja barley it amounted in 1945 to 4230 kg. In 1944 the drought was particularly severe. The results were:

	Yield		Average	Straw- strength	1000-grain weight	Ripening time, days
	1944	1945				
Golden barley ....	100	100	100	3,3	45,1	101
<i>Erectoides</i> 1 .....	88,3	100,1	94,2	4,4	44,5	99,5
44/2 .....	113,9	102,1	108,0	2,4	46,7	105
44/3 .....	90,9	97,5	94,2	9,1	41,1	98,5
44/7 .....	95,2	113,5	104,4	8,5	47,3	101
Maja barley .....		100		3,9	45,1	104
<i>Erectoides</i> 12 ....		99,2		8,6	45,5	104
» 13 ....		95,5		10,0	43,6	104
» 16 ....		91,3		10,0	46,3	103
44/18 .....		98,5		8,6	45,9	103

The late, tall mutant 44/2 seems especially adapted to the conditions at this station. A notable feature is the high yield during the dry summer of 1944 (p. 64). The large-kernelled mutant 44/7, out of Golden barley, attained a higher yield in 1945 than Maja barley, but in 1944 did not come even up to the level of Golden barley. Two Maja mutants are very close to their mother in yield (*erectoides* 12, 44/18).

The high straw-strength in some of the mutants is of considerable

interest. In the year 1945 there were heavy falls of rain, which caused serious lodging. Exceptionally straw-stiff were two erectoid mutants out of Maja barley, these being given the highest possible value, 10, as against only 3,9 for the mother-line, as well as the waxy peculiar mutant out of Golden barley, 44/3, with the relative value 9,1 against 3,3 for the maternal line. Two physiological mutants 44/7 and 44/18 surpassed even the parents.

*Västernorrland station.* — This station is the plant-breeding centre of central Norrland and is as a general rule characterized by a short, relatively dry summer (though much rain fell in 1945). Yield trials have been laid down since 1943.

1943: Preliminary trials. Plots only 1 m<sup>2</sup> in size, one plot of each number. Three Golden-barley mutants were tested, viz. *erectoides* 1, waxy 44/3 and a deep golden-yellow chlorophyll aberrant 42/325, which in two annual yield trials at Svalöf gave 10 % below the mother-line.

1944: This year large machine-sown series were laid down. A. *Erectoides* 1, 44/3 and »late, tall» 44/2 were tested. The plot size was 13,95 m<sup>2</sup> and the number of replicates four. The manuring consisted of 50 kg of superphosphate, 50 kg of potash and 150 kg of saltpetre per hectare. B. The three mutants of the 1943 trials were sown in preliminary series. Plot size: 13,95 m<sup>2</sup>, one plot of each. Manuring: 100 kg of superphosphate and 100 kg of potash per hectare.

1945: Three machine-sown series. A. Four mutants out of Golden barley. Plot size, 11,5 m<sup>2</sup>. Four replicates. Manuring 50 kg. of superphosphate, 50 kg of potash, 50 kg of saltpetre per hectare. B. Four mutants out of Maja barley. Plot size 7,5 m<sup>2</sup>, four replicates. Manuring as for preceding. C. Two mutants out of Golden barley, material grown at the branch since 1943. One plot of each, 13,5 m<sup>2</sup>.

The yield for Golden barley was 4430 kg/hectare in 1944, 3830 kg in 1945; for Maja barley 4530 kg in 1945. In normal cases the maternal varieties are too late for this area and consequently unsuitable for cultivation on a large scale.

	1943	Yield			Average 1944 + 1945	Straw- strength	1000- grain weight	Ripe- ning time, days
		1944	1945					
		A	B	A + B	C			
Golden barley.. (100)		100	100	100	100	100	2,3	43,2
<i>Erectoides</i> 1 .. (106,9)		100,7	119,7	97,4	100,0	104,5	4,4	40,8
44/3 .....	(111,5)	104,7	111,8	100,5	90,6	101,9	7,9	40,2
44/2 .....		104,7		103,7		104,2	1,5	43,8
Maja barley ..				100			1,8	45,0
<i>Erectoides</i> 12..				98,2			1,4	45,7
» 13..				96,9			3,1	43,6
» 16..				93,2			3,0	48,4
44/18 .....				95,4			1,8	46,6

All three Golden-barley mutants showed up better than the maternal line in respect of yield. Especially valuable is the high straw-strength of *erectoides* 1 and 44/3 accompanied as it is by earlier ripening (and earing). The late mutant 44/2 attains a strong vegetative development at the branch station and becomes lodged to a serious extent.

The previously mentioned golden-bright chlorophyll aberrant 42/325 out of Golden barley deserves special mention here. At Svalöf it gives a low yield (10 % below Golden barley). At Lännäs it gave a relative figure of 119,5 in 1943 and a yield of 130,3 in 1944, i. e. very high figures. It eared and ripened in 1944 about a week before the mother-line. Unfortunately, uniform seed from the mother-line and the mutant were not sown in the year 1945. Thus, there is no reliable value for 42/325 for that year. The above-cited relative figures are suggestive of an altered physiological adjustment, presumably connected with the long length of the day in the area concerned.

The yield of the Maja mutants is somewhat lower throughout than that of the maternal line. *Erectoides* 16 also combines here great earliness, increased straw-strength and higher 1000-grain weight.

An interesting feature is that both Maja barley and its mutants are weaker in the straw than Golden barley and its mutants, a condition directly opposite that applying in South Sweden. A tendency in the same direction was already observed in the Ultuna material. The waxiness mutant 44/3 is very straw-stiff, in 1945 straw-stiffer than all other material sown in a similar manner.

*Luleå station.* — This branch, lying as it does at 66° N. lat., only 65 (English) miles south of the polar circle, has a short (although rather mild) period of vegetation. As a rule the rainfall is sufficient. The summer of 1945 was unusually long and hence the examined X-ray mutants, as also their mother-lines, were able to set ripe seed. The early six-row barley Vega was included in the trials.

The size of the plot was 6,4 m<sup>2</sup> and the number of replicates three. Manuring consisted of 300 kg of potassium nitrate, 300 kg superphosphate and 100 kg potash per hectare. The yield for Golden barley amounted to 2570 kg per hectare, for Maja barley to 3450 kg. The material of Golden barley and its mutants was derived from the 1944 cultivations at Lännäs, the material of Maja barley and its mutants from Svalöf.

Luleå presents a repetition of the results at Lännäs concerning the inverse relation of the straw-strength of Golden barley and Maja

	Yield	Straw- strength	1000-grain weight	Ripening time, days
Golden barley . . . . .	100	5,3	49,1	94
<i>Erectoides</i> 1 . . . . .	92,2	6,7	47,1	96
44/3 . . . . .	82,9	9,0	44,2	92
Vega barley . . . . .	87,5	8,0	43,4	80
Maja barley . . . . .	100	5,0	53,5	94
<i>Erectoides</i> 12 . . . . .	103,2	6,7	51,7	98
» 13 . . . . .	91,6	7,3	47,9	91
» 16 . . . . .	97,7	7,3	54,7	93 (?)
44/18 . . . . .	101,7	5,0	53,7	91
Vega barley . . . . .	88,1	8,0	45,9	79

barley as well as their mutants. The waxiness mutant 44/3, in which the earliness is also increased, is the straw-stiffest of all the mutants and substantially straw-stiffer than the maternal lines. Vega barley, however, ripens almost a fortnight before. In yield the Maja mutants show up rather well. The figures however are almost only of curiosity interest.

## 2. SOME QUALITY CHARACTERS OF THE VITAL MUTANTS.

To meet all demands for direct practical value a vital mutant must not only have a high yield but also possess good 1000-grain and hectolitre weights as well as good straw-strength. If a mutant is to be of practical value, it should in addition have, used as feeding barley, a high protein content, used as malt barley, special malting properties.

The content of protein (or starch) has in the course of years been determined for seventeen mutants out of Golden barley and for eight mutants out of Maja barley (crude protein in % of dry matter). Golden barley itself has shown a protein content of 12,3 % as against 10,6 for Maja barley in five annual yield trials. The Golden-barley mutants group themselves round their mother-line, the Maja mutants round theirs. Reduced in proportion to the average value of the mother-lines, the mutants are distributed as follows:

	9,45-9,95	10,45-10,95	11,45-11,95	12,45-12,95	13,45-13,95	14,45 %	M
Golden barley				X			12,3 %
Mutants	1	3	4	1	1	3 2 = 17	12,3 %
Maja barley		X					10,6 %
Mutants	2 2	3	—	1		= 8	10,5 %

The above figures show very clearly that distinct group differences exist. No mutants have arisen with a specially high protein content

concomitant with a high yield. Among the Golden-barley mutants the best is then the peculiarly golden-bright mutant 42/325 with a 10 % lower yield at Svalöf but at the same time a protein content of 15,1 % as against 14,0 % for Golden barley (1940—1941). The best among the Maja mutants is *erectoides* 16 with a 5 % lower yield but a protein content of 10,3 % as against 9,3 % (1944).

In the mutant material examined there exists a distinct correlation between high yield and low protein content. Putting the yield of the maternal lines at 100 and their protein content at 10,0, the relative values for the mutants will be:

Rel. yield	65	75	85	95	105	115
Rel. amount of protein . . . . .	11,60	10,83	10,31	9,71	9,70	
No. of mutants . . . . .	1	3	5	15	1	= 25

Respecting the starch content there is not much to say. High protein content is usually correlated to low starch content and *vice versa*.

On material from the 1944 and 1945 yield trials the malting quality was determined in eighteen mutants, six of which were morphological mutants and twelve physiological. These mutants have a yield level with or above that of the maternal lines. They represent a one-sided selection from the point of view of quality also. The evaluation was carried out by A. B. Stockholms Bryggerier under the direction of Mr. H. THUNÆUS, Engineer. Mr. THUNÆUS has published a report of the results (1946), which has been taken as a base for this survey.

Each sample was divided into halves and malting tests made on each half. The malt was analysed with regard to the extract content of fine and coarse grist, saccharification, vitreosity, and so on. In addition, determinations were made of the wort nitrogen in percentage of the total nitrogen in the malt, the diastatic power as a measure of the  $\beta$ -amylase content and the starch liquefying power as a measure of the  $\alpha$ -amylase content. With the aid of the malting loss and the extract content of the malt the extract production was then determined in percentage of the steeped dry matter of the barley. In a malting barley variety of the first rank — according to the stipulations of the Swedish breweries — the soluble nitrogen must be above 38 % and the fine-coarse difference under 1,1 %. The diastatic power should be higher than 200 units.

TABLE 8. *The mulling quality of the eight best mutants in Golden barley. (After THUNÆUS, 1946.)*

Variety (Cf. Tables 6 and 7)	Protein per cent in dry barley (N × 6.25)	Extract in malt, fine grist, on dry basis	Extract diff. between fine and coarse grist	Wort N per cent of malt nitrogen	Diastatic power in malt on dry basis	Starch liquefying power on dry basis	Extract produced in malt % of barley dry subst.
<i>Erectoides</i> 1 .....	10.4	78.1	1.5	33.7	169	233	71.7
<i>Erectoides</i> 7 .....	10.7	77.7	1.0	32.1	175	234	71.5
44/2 .....	9.7	78.1	0.7	36.1	174	239	71.5
44/3 .....	10.5	77.5	1.1	33.7	145	215	71.4
44/7 .....	10.4	78.0	1.3	39.1	209	305	71.2
44/9 .....	9.7	79.2	0.9	38.2	199	286	72.7
44/14 .....	9.8	78.8	1.4	33.5	203	261	72.6
44/23 .....	10.4	79.0	1.1	36.8	237	319	72.5
Golden barley.....	11.2	78.3	1.1	33.8	177	245	71.3
Significance of variety differences							
P .....	—	0.001	—	0.001	0.001	0.001	—
Smallest significant difference	—	0.37	—	1.82	4.9	13.5	—

TABLE 9. *The malting quality of ten mutants in Maja barley. (After THUNBERG, 1946.)*

Variety (Cf. Tables 6 and 7)	Protein per cent in dry barley (N × 6.25)	Extract in malt, fine grist on dry basis	Extract diff. between fine and coarse grist	Wort N per cent of malt nitrogen	Diatase power in malt on dry basis	Starch liquefying power on dry basis	Extract produced in malt % of barley dry subst.
<i>Series 1.</i>							
<i>Ereoides</i> 12 .....	9.8	78.7	1.1	36.2	271	348	72.4
<i>Ereoides</i> 13 .....	9.8	78.9	1.1	36.7	287	350	72.2
<i>Ereoides</i> 16 .....	9.5	78.3	0.9	38.6	261	347	71.8
Bright green 2 .....	9.3	78.5	1.6	36.4	255	340	71.9
44/18 .....	9.9	79.2	1.3	36.8	243	357	73.0
44/30 .....	9.7	78.2	1.3	36.7	261	334	72.2
44/31 .....	10.2	77.5	1.2	36.2	306	394	71.5
Maja barley .....	9.4	78.5	1.3	38.5	269	360	72.0
Significance of variety diff. P .....	—	0.05	—	0.05	0.001	—	—
Smallest significant difference	—	0.69	—	1.57	11.4	—	—
<i>Series 2.</i>							
44/76 .....	9.3	80.1	1.2	39.4	284	379	73.5
44/89 ped. 1 .....	9.5	79.5	1.0	39.2	320	418	72.5
ped. 2 .....	9.6	79.4	0.8	39.9	313	420	72.2
ped. 3 .....	9.1	79.9	0.7	40.7	280	377	73.2
44/91 .....	9.8	79.0	0.9	39.3	160	248	72.6
Maja barley .....	9.0	80.2	0.9	40.2	289	377	73.5
Significance of variety diff. P .....	—	0.05	—	—	0.001	0.001	—
Smallest significant difference	—	0.67	—	—	28.7	25.2	—

The figures submitted below have reference only to year 1945.

In the morphological mutants tested (Tables 8 and 9) the malting properties have only been slightly altered, in some cases in a weakly negative direction, in one case in a positive direction. This is the more remarkable as other physiological properties have to some extent been considerably displaced. Probably the tested variants represent the very best both in yield and malting quality. Under all circumstances it is incontestable that morphological mutants may signify an improvement in straw-strength and earliness simultaneously with retention of the yield and kernel quality of the original lines.

The group differences are very distinct, especially as regards the diastatic and the starch liquefying power.

Of the physiological mutants out of Golden barley (Table 8), one (44/3) is signalized by a wretched quality. One mutant (44/2) is practically identical with the mother line, possibly somewhat improved with respect to the quantity of nitrogen in the wort. The mutant 44/14 seems to be slightly better, in any case as regards diastatic power. A decidedly superior mutant in the trial of 1945 is the broad-leafed 44/9, which on account of its lateness and its feeble straw has no immediate practical value.

There remain the two mutants 44/7 and 44/23. Both are distinguished by considerably increased kernel size, the former also by a higher yield. At the same time the malting quality has risen considerably, especially in diastatic power and dextrinizing ability. The values here lie far outside the limits of error. The greatest interest attaches to 44/23. In external qualities this is an image of its mother-line (p. 32). The straw-strength is exactly the same, and so is the yield. It would therefore seem justifiable to assume that the improved malting quality in this case is directly due to the increased seed size. From the same  $X_1$  plant there arose, besides this highly interesting type, three worthless mutants, a winter-barley like mutant (44/65), a low-yielding straw-stiff mutant (42/13), and an *albina* mutant.

Golden barley represents an original stage in respect of malting properties. Hydrolyzing enzymes certainly occur rather abundantly in this barley as compared with many other land varieties. By means of crossing and recombination varieties have been produced in which these enzymes are elaborated in far greater quantities. Among these are Maja barley and its still better sister-varieties Kenia and Opal. It is therefore rather probable, as THUNÆUS has suggested, that these strains are less liable to mutate further than Golden barley.

In a Maja mutant (44/91) with long yellowish-brown ears and weak straw the quality has deteriorated disastrously. It falls even below the Golden-barley level so far as diastatic power and dextrinizing ability are concerned (Table 9). Despite this, its 1000-grain weight is enormous (1945: 11 and 12 % higher than that of Maja and Golden barleys) and it yields substantially more than both these strains.

Three mutants (44/18, 44/30, 44/76) are roughly equal in quality, the two first-mentioned being possibly somewhat inferior as regards wort nitrogen and diastatic power.

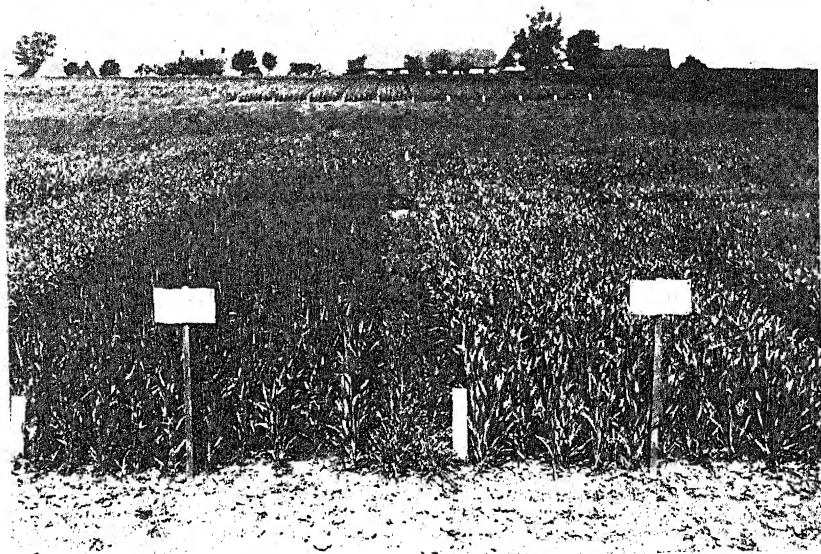


Fig. 11. The extreme difference in earliness between *erectoides* 16 (to the left) and its mother strain Maja barley (to the right). Photographed on June 22, 1946. Yield experiments.

The late mutant 44/31 seems to be definitely superior in regard to diastatic power. Strangely enough it has a low 1000-grain weight. The mutant 44/89 seems to be clearly positive. On an average it gives a 10 % higher yield than Maja (1945) and at the same time shows a distinct improvement of the diastatic power in two of the three pedigrees examined. The 1000-grain weight is almost exactly the same. If these results hold on re-examination, it will be possible, even in so eminent a variety as Maja barley, to induce mutants that improve the quality and simultaneously increase the yield.

The weather conditions affect the malting results in a high degree. The trials reported above must therefore be judged with due caution.

### 3. RELATION BETWEEN THE YIELD OF THE MUTANTS AND THE $X_1$ FERTILITY.

The mitotic injuries increase in frequency and strength in the measure the X-ray irradiation is intensified. At the same time the chromosome breaks increase in number (with translocations and inversions as a consequence), the re-arrangements become grosser, the gene mutations increase. Those seeds which after being X-rayed give rise to the most sterile  $X_1$  plants should therefore contain the highest number of deficiencies, inversions and translocations. Gene mutations which cause visible changes in the phenotype are consequently often combined with gross structural re-arrangements, in the same or in other chromosomes<sup>1</sup>.

The results seem to show that some connexion exists between the yield of the extracted mutants and the fertility of the corresponding  $X_1$  plants. The fewer chromosome aberrations in the irradiated seeds the greater is the general fertility in the  $X_1$ ,  $X_2$  and later generations and at the same time the higher is the yield of the isolated mutants.

A distinct even if feeble positive correlation can be demonstrated for the morphological as well as for the physiological mutants, whether they have arisen from Golden barley or from Maja barley. The  $X_1$  fertility is known for seventeen morphological and twenty-nine physiological mutants, which have in addition been tested in one or several annual yield trials. For the whole material the regression coefficient ( $b$ ) amounts to 0,58, which implies that the fertility increases by 0,6 % for every one per cent increase in the yield. The correlation coefficient ( $r$ ) is 0,32 and its  $P$  value 0,02.

The tested mutants out of Golden barley show a stronger correlation ( $r = 0,52$ ,  $0,02 > P > 0,01$ ). For the Maja mutants  $r$  is only 0,11 and  $P > 0,20$ . The low correlation coefficient for the last-mentioned is probably to some extent due to the average fertility having fallen more profoundly in the Golden-barley material than in the Maja series, with a more powerful interference in the genotype as a consequence. But it may also be associated with the fact that the yield figures for the

<sup>1</sup> Theoretically, mutant individuals that are phenotypically identical but genotypically dissimilar may be isolated from a segregating  $X_2$  generation. Different pedigree lines can therefore give different yields, depending upon whether they are afflicted with deficiencies and lethals, or are quite free from such.

Maja mutants are not yet entirely reliable. The general course of events is however the same.

Physiological mutants, taken separately, give a P value of 0,05, morphological mutants a P value greater than 0,05. The probable existence of a correlation in the last-mentioned as well is shown by the following survey.

		Fertility of the $X_1$ plants in percentages									
		10	20	30	40	50	60	70	80	90	100
Yield for Golden mutants, Golden	= 100 .....	80				83	89	89	83, 92	91, 98	
Yield for Maja mutants, Maja	= 100 .....								51, 76, 95	95, 102	97, 100

The occurrence of a correlation is further confirmed if the mutants are divided into two groups, one comprising mutants equivalent to or better than the maternal lines (relative values 95—115) and the other containing mutants that are inferior to the mothers (relative values below 95), and the average  $X_1$  fertility is then determined for each group.

		Average $X_1$ fertility	D/m	n	P
Morphological mutants	Equivalent or better	$84,00 \pm 4,15$ %		6	
"	Inferior .....	$66,65 \pm 6,20$ %	2,33	11	$0,05 > P > 0,02$
Physiological mutants	Equivalent or better	$76,93 \pm 4,32$ %		14	
"	Inferior .....	$65,27 \pm 5,85$ %	1,61	15	$0,2 > P > 0,1$
Total	Equivalent or better	$79,50 \pm 3,43$ %	2,48	20	$0,02 > P > 0,01$
"	Inferior .....	$65,77 \pm 4,00$ %		26	

According to this method of computation the difference within the morphological group is rather considerable, but is less prominent within the physiological group.

Of the eleven mutants in the morphologically drastic group, which in general show a low  $X_1$  fertility, only two have so far been tested in yield trials. They were conspicuously inferior. The rest set little seed. If they had been tested in yield trials also, the correlation coefficient would undoubtedly have increased considerably.

If this view is correct, the plant-breeder who wishes to employ mutations in his work must seek the middle way between two extremes, (1) he ought to produce so great a number of vital mutants as possible, (2) he ought to exclude the lethalizing effect that appears after high

X-ray doses and high  $X_1$  sterility. This difficulty was already stressed by STADLER in 1930.

#### 4. CONCLUSIONS.

Among morphological mutants out of Golden barley, *erectoides* 1 and 7 were tested in yield trials over a long series of years, the former also at some of the branch stations of the Swedish Seed Association. In fifteen series of trials (each with several replicates) it gave a relative yield of 101. It shows a substantially increased straw-strength and may be denoted as a progressive variant.

Maja barley has the highest yield of all the sufficiently tested South-Swedish strains. In six series *erectoides* 12 gave a relative value of 100 and showed a considerably increased straw-strength. *Erectoides* 16, with better straw-strength and a considerably higher 1000-grain weight, gave — in spite of its increased earliness by six or seven days — a relative value of 95 in six trial-series. Both these mutants may be denoted as progressive. The same applies to *erectoides* 13 with its enormous straw-strength.

Among the physiological mutants out of Golden barley special mention may be made of a »late and tall mutant», (44/2), which was studied in eleven trial-series and gave the relative value of 104. The high yield is probably associated with the longer and kernel-richer ears. Its ecological requirements seem to be changed. The straw-strength is about the same. A very waxy, peculiar mutant (44/3) was awarded the relative value of 99 in twelve series, and belongs to the straw-stiffest observed in the Scandinavian assortment. Unfortunately, its 1000-grain weight is very low. Mutant (44/7) is extremely high-yielding, with an exceedingly high 1000-grain weight (more than 10 % above that of Golden barley). In six trial series it gave the relative figure 109, thus ranking almost level with the Maja barley.

Of still greater interest, of course, are the best physiological mutants out of Maja barley. In five series the mutant 44/18 gave the relative value of 99, but, moreover, is considerably straw-stiffer and ripens quicker. The best, however, seem to be the mutants 44/89 and 44/76, which, to be sure, were only tested in the year 1945 but in several pedigree series were then about 10 % above Maja barley. In addition, 44/89 has a more copious tillering, a higher kernel number per ear, and ripens earlier than the mother-line.

The highest yielding mutants were also investigated with respect to their malting properties. In some, the diastatic activity ( $\beta$ -amylases) and starch liquefying power ( $\alpha$ -amylases) had been considerably reduced. In Golden barley with its relatively low content of decomposing enzymes valuable variants seem able to arise comparatively easily. The most interesting is a mutant that reproduces perfectly the Golden-barley type in outer respects but deviates by somewhat broader leaves and an essentially increased 1000-grain weight. The last-mentioned quality is accompanied by a considerably improved malting quality. This is also the case in the large-kernelled mutant 44/7, which in addition produced a rich yield. The highest producing mutant from Maja (44/89) likewise seems to possess improved malting properties.

Finally, it is shown in this Chapter that a weak but distinct correlation exists between on one hand the  $X_1$  fertility of the mutated original plants (a measure of the X-ray effect) and on the other hand the yield of the pure-bred mutant, in

conformity with STADLER's assumption (1930). A mutation with a positive action is often accompanied by mutations having lethal effects.

## VII. MUTANTS IN OTHER AGRICULTURAL PLANTS.

### 1. WHEAT.

*Earlier results.* — In the diploid species *T. monococcum* and *aegilopoides* (SMITH, 1936, 1939) spontaneous as well as induced chlorophyll aberrants are readily formed. These are rare in the tetraploid stage and almost never occur in the hexaploid. FRÖIER (1946) has especially stressed the different facility with which chlorophyll mutants arise in hexaploid oats and hexaploid wheat. The hexaploids of the two genera are probably genomically differently constructed.

In wheat polyploids also, however, a couple of cases of chlorophyll aberrants have been observed, especially after crosses between tetraploid species among themselves and between tetraploids and hexaploids (FRÖIER, l. c.). An undoubted chlorophyll mutation, spontaneously arisen, was observed by NEATBY (1933) in hexaploid wheat. It was of *virescens* type, i. e. white as seedling but turned green later and could even produce seed in the homozygous state. This *virescens* type proved to be instable, for it mutated (1) back to the normal state, (2) to pure *albina*, and (3) to a second *virescens* type with considerably slower chlorophyll formation. SCHWARNIKOW described (1936 a, p. 477) a pale-green normally fertile mutant, which may belong to the *viridis* group and which arose in the progeny of heat-treated seed. He likewise found solitary »chlorophyll mutations» in progenies from aged seed (1937).

An important group of lethal or sublethal mutants consists of the so-called speltoids, which were first described by NILSSON-EHLE (1917) and were later investigated by him and a long series of other workers. The speltoids may be divided into A, B and C types, according to disturbances found in the segregation and specific cytological anomalies. Of special value for the whole speltoid problem was WINGE's hypothesis (1924) of the connexion of these mutations with chromosome deviations. UCHIKAWA (1941) has recently published a survey of this region of investigation. The so-called compactoids arise as secondary phenomena in the  $F_2$  and  $F_3$ . The frequency of the speltoid mutations ranges from about 0,1 to 1 %.

SCHWARNIKOW (1937, 1939) has shown that the number of speltoids as well as of sterility mutants, dwarfs, etc. is very substantially increased in progenies from aged seed. Seed that has been stored for about ten years germinates very badly: in twelve samples (9—11 years old seed) of two varieties the germination amounted to 40 %, while the control showed 88 %. In the same material the mutation frequency (calculated on the number of plant progenies) increased to 17 %, the majority of which consisted of lethal and sterile products, especially speltoids.

The same author also found (1936) that temperatures of 40°—60° C. considerably raise the number of mutants in the  $I_2$  progenies. A temperature of 40° for 21—31 days produced an aberrant frequency of 32 %, 60° for 16—26 days a frequency of 45 %. The mutants that were isolated usually possessed a lethal character; they were dwarfs, narrow-leaved variants, speltoids, small-eared and light-green types, etc. But there also appeared vital mutants with increased waxiness, red-brown ears, rather dense ears, and increased straw-strength. The control material, which however was not particularly large, did not give a single aberrant. This method of inducing vital mutants requires further study. It can be definitely asserted that it

has not the same effect in diploid barley, even at higher temperatures. Still, the results are worthy of the greatest attention.

LEWICKI (1922) found in *T. durum* var. *coerulescens* a mutant with »fragile spikes» and hairiness at the base of the spikelets. These properties characterize *T. dicoccoides*, which is regarded as an ancestor of cultivated wheat. In addition, the mutant differs considerably from the parent line by its longer straw and spikelets.

COFFMAN (1924) observed solitary aberrants out of the variety Mindum from *T. durum*, probably arisen through mutation and characterized by supernumerary spikelets in the lower portion of the heads. Similar forms have been described by MEUNISSIER (1918), PERCIVAL (1921) and KAJANUS (1924), but it has not been clarified whether they have arisen by mutation or segregation.

CHRISTIANSEN-WENIGER (1926) found a peculiar mutant in the spring-wheat variety »Green Mountain» with a very wide range of modification, the form of the ear varying from long and spelta-like to dense compactoid, in extreme cases deviating greatly from the average type without there being hereditary differences.

KOLKUNOV (1928; Biol. Abstr. 7, No. 17723) measured year after year the stomatal length in a number of pure lines of wheat. Out of the strain »Banatka», itself rather large-celled, a form with exceptionally large stomata and reduced awns, probably arising by mutation, was isolated in extensive selection experiments. The mutant was to some extent dwarf-like, had a feeble reproductive system, and was sensitive to frost and drought as well as to attacks of saw flies (*Cephus* sp.). KOLKUNOV considers that »a mutation in cell-size may appear in any pure line».

DEKAPRELEVICH (1929), according to Biol. Abstr. 7, No. 343, has observed a mutant with a long lax spike, long rachillas, and coarse wide empty glumes, maturing more slowly than the parent line. He compares the mutant in question with the speltoid type and explains the strong pleiotropism as a result of a group of linked genes having mutated.

X-ray mutants have been produced in diploid species (*T. monococcum*, STADLER, 1929; SMITH, 1936, 1939), tetraploids (*T. durum*, SAPEHIN, 1935), hexaploids (DELAUNAY, 1930, 1931, 1932, 1934; SAPEHIN, 1930, 1934, 1936; FONDARD et CABASSON, 1939; RANJAN, 1940; GUSTAFSSON, 1941 a). Cf. FRÖIER, 1946 a. STADLER produced chlorophyll aberrants only in *T. monococcum* and found there a mutation frequency comparable with that of barley. In the same species as well as in the closely related *T. aegilopoides* SMITH isolated about 400 mutants, 80 of which, i. e. every fifth mutant, were viable in the homozygous form.

According to SMITH, solitary mutants are formed with a strong morphological effect accompanied by high vitality and fertility. To these belong mut. *biaristatum* (*ba-a*): well-developed awn on the lemma of each of the two florets of a spikelet; mut. *compactoides* (*c-1*, *c-2*): rachilla shortened, the mature plant somewhat dwarfish with thick, bristle culms; mut. »awned glumae» (*ga*): both outer glumes with awns, the awn of the lemma reduced, glumes soft, seeds easy-shelled; mut. »wiry» (*wi*): young leaves narrow and stiff, fertile only in green-house cultures. In addition there is the morphological mutant *densinodosum* (*mi*): many and short internodes. The mutant does not develop seed in field cultures, and does so poorly in the green-house. Of very great interest are two earliness mutants (*e-1* and *e-2*), one of which under field conditions is three weeks earlier than the mother-line. The other is not quite so extreme but is more vigorous.

The Russian investigators cited above, who worked with *T. durum* and *T. vulgare*, have attained valuable results. They have generally irradiated growing plants at anthesis. In this way extremely powerful chromosome disturbances are induced that are unmasked in the progeny from the irradiated plants. The  $X_2$  plants are »extremely polymorphic» and in the great majority of cases »characterized by chromosome aberrations» (SAPEHIN, 1936, p. 36). (Cf. also DELAUNAY, 1930). A close positive correlation was found between the degree of nuclear abnormalities and the degree of phenotypic changes.

*T. vulgare* has been carefully studied. DELAUNAY (1934) divides the mutants produced into seven groups: (1) awned forms, (2) speltoids, (3) dense-eared, (4) point-glumed forms, (5) dwarfs, (6) other clear mutants, (7) undoubted mutants that are difficult to characterize. In certain cases an induced mutation frequency is obtained of 60 % as against a spontaneous frequency of 0.1 %, i. e. an increase by 600 times. SAPEHIN (1936) finds minor variations in the form of the glumes and in the density of the spikes, numerous speltoids and semispeltoids, single dwarfs, etc. Spring and winter forms behave similarly.

In SAPEHIN's experiments (1935) *T. durum* gave fewer mutants than *T. vulgare*. There would however seem to exist no fundamental difference in behaviour.

The two Russian investigators lay great stress on the value for plant breeding of the results obtained. »Artificial mutations are becoming a valuable method in plant breeding» (SAPEHIN, 1935). In his comprehensive work of 1934 DELAUNAY expresses himself to the same effect. Whether some of the induced mutants actually obtained practical value in the USSR, is not known. No yield figures have been published. The method of X-raying growing plants certainly gives many mutants but is disadvantageous from other points of view.

GUSTAFSSON (see 1941 b, p. 241) X-rayed in the mid-thirties seed from Pudel wheat (a white-kernelled variety) and obtained speltoids, straw-height mutants, dense-eared straw-stiff types, several of them fully vital to judge from appearances. They have however not been studied further. Pudel wheat is now devoid of any practical value. In this case it can be positively asserted that spontaneous crossing or contamination of seed cannot account for the origin of the variants.

MORITZ-VOM-BERG (1935) described a mutant in *T. vulgare* produced by treatment with acetic acid, and BYNOV (1938) considered that he had induced mutants by means of electric currents.

*New results.* — Experiments carried out jointly by FRÖIER and GUSTAFSSON with a view to producing practically valuable variants by the mutation method have confirmed the results of the Russian investigators. With suitable X-ray doses a mass mutating sets in, not only with regard to speltoids but also compactoids, awn mutants, vital mutants with changed straw-height, straw-strength, earliness, tillering. On the base of the pure line or the homozygotized cross-product there arises a new polymorphy that is not inferior to that of the original land varieties. The survey given below illustrates this (for further details, see FRÖIER, 1946 a, b):

	Number of $X_2$ families	Number of progenies segregating for speltoid mutants	Other mutations
Spring wheat			
Kolben 10.000 <i>r</i>	16	3 = 19 %	Ear density, length of awns
» 10.000 <i>r</i>	207	89 = 43 %	Ear density, length of straw, breadth of leaves
» 15.000 <i>r</i>	33	12 = 36 %	Length of straw, tillering, earliness
Diamond II 10.000 <i>r</i>	100	46 = 46 %	Straw stiffness, length of awns, ear density, earliness
Winter wheat			
Gluten 20.000 <i>r</i>	61	10 = 13 %	<i>Compactum</i> , straw height, ear density, awnness
Scandia III 15.000 <i>r</i>	617	52 = 8 % <sup>1</sup>	<i>Compactum</i> , awnness, straw height, earliness, other properties
» » 20.000 <i>r</i>	427	61 = 14 % <sup>1</sup>	<i>Compactum</i> , awnness, other properties
Controls			
Spring wheat	100	—	No obvious changes
Winter wheat	339	—	No obvious changes

Thus, in certain cases the speltoid frequency amounts to 40—50 %. The number of other mutants is occasionally very high. In all, controls of 439 plant progenies have been examined, and in these not a single speltoid mutant has been observed with certainty. Scandia III is a commercial strain of high value, which is not yet completely uniform. Its variation was slight in the control tests as compared with that of the  $X_2$  material.

Mr. J. MAC KEY, who has taken over the genetic and cytologic analysis of the numerous speltoid and compactoid cases, has made a further inventory of some of the  $X_2$  material in Scandia III (20.000 *r*). In fifty  $X_2$  families ten (20 %) were observed that contained speltoids, four (8 %) contained compactoids (partially modifications?), and thirty-one contained single or numerous long-eared types. Several of the last-mentioned are certainly distinguished from the normal type. Awn mutants and sterile forms have also been isolated.

<sup>1</sup> Preliminary calculation.

Although yield trials and quality analyses have not yet been carried out, it is clear that the wheat-breeder can produce vital mutants of practical value by means of X-ray treatment. The mutants appear in flocks to a larger extent than in barley, probably owing to the fact that the barley mutants are often derived from point mutations, while the wheat mutants are associated with chromosome aberrations, point mutations here only exceptionally giving a phenotypic effect. FRÖIER, GELIN and GUSTAFSSON (1941) found, after 20.000 *r*-units, no fewer than 94 % disturbed cell-divisions in the germinating seed of *T. vulgare*. Chlorophyll mutants of *albina* type have never been observed, although solitary ones of *viridis* or *alboviridis* type have. According to GUSTAFSSON, these are to a large extent due to chromosome disturbances.

## 2. OATS.

*Earlier results.* — Spontaneous chlorophyll mutants arise now and then also in the hexaploid *Avena sativa* (see Table 1 in FRÖIER, 1946 a). Both plastid and chromosome mutations are known, the latter usually being of *albina* or *viridis* type. Of special interest is ÅKERMAN's investigation of a sublethal *lutescens* variant (1922). The monoheterozygote can here be distinguished, while di- and tri-heterozygotes have a normal appearance. This chlorophyll aberrant, however, was not formed by mutation but after a cross between a monofactorial and a bifactorial line. The *chlorina* type described by ÅKERMAN and FRÖIER (1941) arose, on the other hand, as a mutation.

More or less sterile dwarfs arise not so infrequently (WARBURTON, 1919; STANTON, 1923; GOULDEN, 1926). STANTON observed both a recessive and a dominant dwarf. ZHEGALOV (1920) found a peculiar mutation that caused giant growth.

The so-called fatuoid mutants must be denoted if anything as sublethal. They were first described by NILSSON-EHLE (1911 a), who also showed that the heterozygote is intermediate and referred it in 1921 to the category of complex mutations. HUSKINS (1927, 1933) divided the fatuoids, in agreement with the speltoids in wheat, into different groups and considered them to have arisen as a consequence of chromosome aberrations. JONES (1930), who also isolated so-called subfatuoids, approached NILSSON-EHLE's view. The repeatedly advanced opinion that the fatuoids were cross-products between *A. sativa* and *fatua* has been definitely refuted (ÅKERMAN and BADER, 1938).

Far more important from the point of view of vitality are the kernel-colour mutants. These were also first described by NILSSON-EHLE (1911 b). In a seed-parcel of 700 kilograms of black oats he found 2.891 deviating kernels, 2.774 of which were grey, 115 white (and 2 yellow). Most of the grey and white kernels had probably arisen through mutation. ÅKERMAN and FRÖIER (1941) found one mutated kernel per 2.000 kernels in the widely cultivated black-oat varieties Engelbrekt II and Stormogul. The two commercial strains mentioned are monofactorial with regard to the black colour. By a successive transference of the cultivated varieties that are monofactorial to bifactorial, the number of white and grey kernels in the seed is

A sporadic morphological mutant was described by COFFMAN and QUISENBERRY (1924). It arose out of Burt oats (*A. byzantina*) and showed very strong pleiotropism. The kernels were partially naked; 40–50 % of the caryopses were hulled by the threshing. In addition, the glumes were long, the spikelets 2–6-florate with elongated rachillas, causing the florets to extend beyond the glumes, and the ripening time shortened by several days.

According to MARTINET (1928), a peculiar variant, called »soldanelle», arose as a mutation out of a Swiss strain of oats. It is characterized by yellow kernels and is very early. The strain in question formed, in fact, »many mutations». The same variant also arose after a cross between the Swiss strain and a Canadian variety.

DERRICK and LOVE (1937) X-rayed a dwarf form of oats. From 332 caryopses there arose eleven fatuoids of dwarf type.

Of great interest is the different mutability which FRÜIER found in hexaploid commercial strains after X-ray treatment.

Stormogul	gave	12	genetically	controlled	chlorophyll	aberrants
Victory	}	»	1—3	»	»	»
Golden Rain						
Clock II						

Stormogul	}	gave	12	genetically controlled chlorophyll aberrants			
Abeds Nova							
Sapeli		»	0	»	»	»	»
Engelbrekt II							

This difference seems to be due to unequal reduplication of chlorophyll-determining chromosome parts.

Induced fatuoids have not arisen at all in the Svalöf experiments. Nor has the frequency of the spontaneously so numerous kernel-colour mutants been noticeably increased.

*Vital mutants* have been obtained in *A. strigosa*, *A. brevis* and *A. sativa*. *A. strigosa* is cultivated very little in Europe, and then in dry and poor soils in northwestern Germany as well as in certain parts of Great Britain and Ireland, and hence it has but little economic value. Several mutants were isolated out of a line of this species, among others a form with a silvery colour on lemma and palea and a lower culm (mut. *argentea*, according to FRÖIER) and a substantially earlier, more rapidly withering form, likewise with a shorter culm. Further, physiological mutants that merely altered the straw-height (in a positive or negative direction) or tillered to different extents were not uncommon (FRÖIER, 1946 b).

In *A. brevis* there were observed mutants in straw-height, ripening time (a striking earliness mutant) as well as a mutant with the phylogenetically important property that the spikelets (the fruits) loosened on ripening.

In *A. sativa* mutants have been more especially obtained from the black-oat variety Clock II. This arose at the beginning of this century as a spontaneous cross between Golden Rain (white-kernelled) and Clock (black-kernelled). The length of the culm is rather easily influenced, positively as well as negatively. Awn mutants have been isolated. Different earliness mutants have been observed, one of which in comparative cultivation was specially striking. Besides the last-mentioned mutants, which have practical value, types have also arisen that ripen very late, up to fourteen days after the original line. Different gradations in lateness occur. In Stormogul, a black-oat line already isolated in 1889, solitary straw-height mutants have been induced. But this as well as the white-kernelled Victory oat, which has given a couple of lateness mutants, are more stable than Clock II with regard to vital aberrants (cf. Golden barley, Maja barley, Ymer barley, p. 49).

The results in hexaploid oat are very promising for future mutation work. FRÖIER has carried out a large number of crosses with the best earliness mutants.

### 3. FLAX.

During several years irradiation experiments have been carried out on fibre and oil flax (GRANHALL). The suitable X-ray doses are between 30,000—40,000 *r*. In the  $X_2$  and  $X_3$  generations strong selection has been carried out for tall and for short straw, high and low seed-content. Such a selection leads to tangible results. It has however been found that the spontaneous crossing frequency is rather high, and that pure lines grown next to one another gradually become heterogeneous as a consequence of hybridization. The results of the X-raying were therefore not unexceptionable, and the experiments had to be commenced afresh. No cases of chlorophyll mutations have been observed.

Such have however been demonstrated by LEVAN (1944). In the progeny from irradiated seed of diploid Hercules and Concurrent three mutants arose, two of which were lethal or sublethal. Cultivation of the third mutant (out of Concurrent) on a large scale has given extremely interesting results. In colour this mutant is yellowish-green and grows more slowly than normal plants; eventually, however, it attains the same height and sets abundant seed.

GRANHALL (1946, Table 1, p. 296) has found that the mutant is fully vital, in spite of the fact that it must be denoted as a chlorophyll aberrant (cf. mut. 42/325 in barley, p. 68). Its yield, so far as straw production is concerned, is above that of the mother-line. The seed-yield is about the same. At the same time, however, the content of long fibre is higher.

	Straw yield kg/hectare			Seed yield kg/hectare			1000-grain weight
	1944	1945	Relative yield	1944	1945	Relative yield	Average gr.
Concurrent . . . .	5330	4570	100	1680	1350	100	5.6
Mutant . . . . .	5630	4850	105.9	1750	1290	99.9	5.3

The mutant has a somewhat lower 1000-grain weight. The unaltered seed-crop ought therefore to be due to a higher number of capsules per plant or more seeds in each capsule.

Flax is of very great interest from a mutation point of view. At about the same time as BAUR, TINE TAMMES (1925) framed her hypothesis on the evolutionary importance of »Kleinmutationen». In the light of the rather high frequency of spontaneous crossing it is possible that many of the variants that she studied depend on segregation rather than on mutation.

#### 4. SOME OTHER KINDS OF PLANTS.

A few words may be awarded to mutants out of soy-beans (ANDERSSON, 1944, p. 291). On account of the poor weather conditions in the years 1940—1942 a large part of the original material was ruined. Still, several interesting mutants have been obtained. Some may be denoted as morphological, for instance, those with another seed-colour, presence of anthocyanin, entirely new mode of growth. Physiological mutants have also arisen, though none unfortunately show so advanced an earliness as the corresponding types in barley and oats. The work is going on to a large extent. Two X-ray selections out of Altonagaard AI gave in the 1944 and 1945 trials the relative figures 116 and 108 as regards yield, and ripened three and two days earlier.

Extensive trials have been carried out on oil-turnips and mustard. These are not entirely self-fertilizing. From the common spring variety Regina oil turnips a homogeneous line has been obtained which ripens some days earlier and grows much faster in seedling stage. This is one reason it is not so severely attacked by flea-beetles. This new variety has been tested in a great number of yield trials in southern Sweden. In Svalöf its relative yield lies at 105 (mean of 1944 and 1945). In five other places its yield averages 111. Its content of fats is 39,3 % against 38,6 % for the original strain. *No similar variety has ever arisen in pedigree cultures.* Its striking habit of growth makes an origin by means of mutation highly probable.

In mustard several mutations have been isolated, some of them influence flower colour (different gradations from yellowish to white). Some X-ray pedigrees have increased yield considerably (44/113: rel. yield 113, 44/156: 109). A great number of lines have been tested. Their origin by means of mutations cannot be definitely stated. From a practical point of view this is of course immaterial.

Full accounts will be published by Dr. ANDERSSON.

Some interesting mutants have been isolated also in sweet-lupine. Data concerning these will be published later on by Dr. TEDIN.

## VIII. GENERAL SURVEY OF THE RESULTS.

*Mutation types.* — In cereals spontaneous and induced mutants may be conveniently divided into three groups: (1) chlorophyll mutants, (2) sterility and lethality mutants of different kinds, and (3) vital mutants. The chlorophyll mutants are so characteristic that they should strictly be kept separate from the sterility and lethality mutants. Spontaneous and induced chlorophyll mutants are common in diploid organisms but become rare in the polyploid. This applies especially to *albina* types. In polyploid wheat and oat species speltoid and fatuoid mutants take the ascendancy instead.

The vital mutants are characterized by normal fertility and show no noteworthy lethality in the vegetative stage. They may conveniently be divided into a morphological and a physiological subgroup. The yield is usually more or less reduced. In barley the so-called erectoid mutants are especially striking.

*Mutation frequencies.* — From the extensive series of experiments laid down in diploid barley it seems evident that pure lines have a very high stability. On the other hand, the mutability rises considerably in cross-strains, even in such as are homozygous judged from external signs. It is possible that minor structural aberrations still occur and that these cause irregularities in meiotic pairing. In this way new structural aberrants may be formed, and some of them have a phenotypic effect. It is also possible that the genes are labile owing to the recombination itself, i.e. an unselected genotypical environment.

Even in very stable genotypes the mutation frequency rises considerably if the external conditions are altered, e.g., by heat treatment or old age of the seeds. In the analysed cases the effect corresponds to an X-ray dose of 500 *r*, and is thus rather strong.

One induced morphological mutant in barley — fertile or infertile — corresponds in frequency of occurrence to 22 chlorophyll aberrants, 15 mutants that prevent normal meiosis and gamete formation, and about 30 cases of »translocation sterility». In order to obtain one mutant with a distinctly higher yield the barley-breeder must induce about 700—800 worthless types at the same time.

As a contrast to these figures it may be mentioned that of three mutants in flax which arose in Dr. LEVAN's experiments one proved to be clearly superior to its mother-line. In this case luck and material favoured the mutation investigator.

*Practically valuable mutants.* — The primary object of the investigations described above has been to determine to what extent in-



Fig. 12. Two ears of an extreme mutant in barley, showing long ear internodes and a greatly increased number of spikelets in the fertile rows. Isolated in 1946. Different gradations occur. Mother strain: Ymer 40/13 bz.

duced and spontaneous mutants meet the plant-breeder's demand for cultivating value. In the best cases the yield ought to have risen, the straw-strength increased, the 1000-grain weight highered, the quality

improved. It is however to be regarded as a gain if any one of these can be attained. The plant-breeder then has it in his power to transfer solitary good qualities from otherwise poor mutants to already existing commercial varieties. STADLER was in 1930 rather sceptical as to the possibility of improving cereals by means of induced mutations. The variation at the disposal of the plant-breeder would still suffice, he thought, a good bit forward. That may be so, but in the same year ELISABETH SCHIEMANN pointed out in an overlooked paper that the good straw-strength in a widely spread German malting-barley strain was derived from a mutant inferior in most qualities.

Certain mutants yield more than their mother-line. The yield is undoubtedly a measure of the general vitality, both as regards the vegetative and reproductive phase. Thus, high yield points to increased vitality (under the prevailing conditions of cultivation). The results from the two barley strains most carefully examined here supplement each other. One strain, Golden barley, is an old, pure line, with the same qualities now as when it was isolated. It is manifestly very stable; mutations and spontaneous crossings are rare. Maja barley, the other strain, is not an original pure line in the same sense as Golden barley but a cross-product, which has become gradually homozygotized. Up till now its high yield has scarcely been surpassed by any other varieties of Scandinavian two-rowed barley.

With reference first of all to *the morphological mutants*, some of these attain to the yield of the maternal lines or can even surpass them. This applies especially to certain bright-green mutants, though also to such sharply defined types as *erectoides* 1 and 12. In the two last-mentioned the straw-strength has also risen. Individual properties such as 1000-grain weight and earliness may also change in a favourable direction. Especially remarkable is *erectoides* 16, which ripens one week before the mother-line and has only a slightly inferior yield, just as in fact *erectoides* 13, which is immensely straw-stiff. Mutants can thus be formed which deviate very much while retaining the productive power of the original line. The fully fertile morphological mutants give on an average a lower yield than the corresponding physiological ones.

Out of Golden barley two or three *physiological mutants* have been induced which are superior in yield to the mother-line. One of them (44/7) is 9 % higher. This valuable quality is probably connected with the greatly increased grain weight. Another mutant, 44/2, ripens a little later and is considerably richer in straw. Its yield varies greatly,

depending on the amount of rainfall during the vegetation period. The Svalöf average for six years, however, lies at a relative value of 103.

Still more interesting, of course, are the yield-raising mutants out of Maja barley. Three different pedigree lines from mut. 44/89 have given on an average 10 % more than the mother-line. They resemble the latter in most respects. High-yielding mutants are also 44/76, 44/91 and 44/30, the last-mentioned of which has been tested in rather extensive trials. As the Scandinavian plant-breeders have not succeeded, despite fifteen years of continuous work, in forcing the yield of the barley noticeably above the level of the Maja strain — either by selection or crossing — the best mutants must be assigned very great theoretical and practical interest.

Methodical work must therefore sooner or later lead to a raising of the yield by the mutation method as well, even in the case of the chief commercial varieties. It must not be considered as fundamentally impossible that an induced or spontaneous cereal mutant becomes so worth cultivating that it can immediately replace the original strain. The high requirements exacted by the plant-breeder of every new market variety will presumably make such a result less probable; impossible it is not. (Cf., for instance, the successful variant obtained in oil turnips, which will possibly be brought into the market in 1947.)

The old land varieties were exceedingly heterogeneous, but the value of the strains contained in them varied. On the basis of the commercial strains it is now possible to produce a new polymorphy by means of mutation. The average yield of the tested *fully fertile and vital* physiological mutants lies only about 5—10 % below that of the original line. At the same time, however, the original line has been broken up in many different directions. The straw-strength has sometimes been improved, sometimes deteriorated, sometimes it is *status quo*. The same applies to earliness, 1000-grain weight, malting properties. Such mutants have been described in great numbers. For brewing purposes a mutant out of Golden barley is of special interest. Externally it is a faithful copy of the maternal line, in a mixed crop with this it would not be distinguishable. The yield is exactly the same. The leaves, however, are somewhat broader and the 1000-grain weight higher. In addition, it must be denoted as an entirely new and improved malting variety (THUNÆUS, 1946).

A mutant, suspected already in 1945 and certified in 1946, falls outside the known variation sphere of Scandinavian two-rowed barley with regard to floret and kernel number of the spikes. In the com-

mercial strains the fertile spikelets attain an average number of 24—28 (cf. the similar statement made by ÄBERG and WIEBE, 1946, p. 14, for American varieties). Several progenies of this mutant show a spikelet number up to 36—38, in other instances up to 34—36, i.e. an increase with 30—40 %. If this property can be combined with a higher straw-stiffness (the spikes are very heavy), and possibly some other valuable properties, the mutant may entirely renew Scandinavian barley breeding. It arose from the high-bred strain Ymer b<sub>7</sub>.

Another mutant, *erectoides* 13, seems to have improved the straw-stiffness above the level of commercial barley strains. *Erectoides* 16 most certainly breaks the frame with regard to earliness. Both yield also well. Other mutants have been found that raise the kernel weight considerably and simultaneously improve yield. These examples, better than long statements, illustrate the possibilities involved in methodical mutation experiments.

An interesting case that visualizes the interaction between mutation, yield and environment has been observed in a chlorophyll mutant out of Golden barley. A golden-bright vital aberrant produced at Svalöf yielded 10 % below the mother-line. Cultivated about 1000 kilometres further north, it yielded 25 % above Golden barley in 1943 and 1944 in preliminary experiments. Probably in this case length-of-day conditions played their part, favouring the assimilation. Similar conclusions have been drawn by GRANHALL and LEVAN for a yellowish-green chlorophyll mutant in flax, induced in the foreign strain Concurrent. The seed production is the same, but the straw crop per hectare has increased by 6 % and also the fibre quality has been improved. This remarkable result shows that »defect or loss mutations» may imply direct improvements.

*Morphological effect and vitality.* — According to the results obtained here, the effect of the mutations and the yield of the mutants are intimately connected. This is excellently illustrated by the erectoid mutants. The highest productive types deviate only in a few characters from the maternal line and differ slightly in general habit. When straw-height and ear-length have changed considerably in one or the other direction, or new kernel characters have been induced, the production also falls. The same condition applies to the mutual yield of the three groups of bright-green, erectoid and drastic mutants (GUSTAFSSON, 1946 a). The relative figures average 101, 88 and 84 for the three groups. Moreover, certain drastic mutants are entirely sterile.

*Lethal factors and yield.* — STADLER advanced the assumption in 1930 that solitary good mutations can be produced by X-ray treatment but that at the same time lethal factors are induced in great number. This assumption has been confirmed, although the correlation between the yield of the mutants and the sterility of the original plants (a measure of the lethalization) is not especially strong. Nevertheless, this correlation is rather clear for the mutants out of Golden barley. Most seed-material of this line has been subjected to high X-ray doses and special pre-treatment. By this means a considerable  $X_1$  sterility has been induced. The correlation is weaker in mutants out of Maja barley, where the X-ray effects have been less violent. One case that excellently illustrates STADLER's assumption may be mentioned. Among others, a peculiarly waxy, extremely straw-stiff mutant was isolated out of Golden barley. Its yield — calculated on all trials — is equal to that of the maternal line. The 1000-grain weight is low, however, and the malting properties have deteriorated. Thus, good and bad properties accompany each other. With SCHIEMANN's report in mind, however, the enormous straw-strength may no doubt be assigned some value in future crossing work.

*Control of the mutation process.* — Series of special experiments seem to show that the mutation process can be experimentally controlled. If the cell environment is altered in different ways, or the irradiation effect is varied, this causes quite different mutations and mutant types to arise. For instance, it may be regarded as certain, firstly, that, as opposed to *albinas*, *xantha* mutants are not produced in the progeny of entirely fertile  $X_1$  plants, secondly, that *alboxantha* types preferably arise from irradiated germinating seeds or seeds rich in water, thirdly, that for their origin the so-called drastic mutants require a very strong irradiation effect upon the treated seeds.

The results obtained so far at Svalöf and abroad are certainly rather meagre. Continually extensive experiments are being carried out, however, with the express purpose of elucidating these questions from a practical point of view.

In conclusion stress must once more be laid on what has here been adduced respecting the yield of the vital mutants, viz. that the results in diploid barley seem to apply to all agricultural plants so far examined, whether they are diploid or polyploid. A methodical work will sooner or later lead to positive results. The plant-breeder cannot

neglect the artificially induced mutants for the further improvement of his varieties.

Svalöf, June 1st, 1946.

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# A CONTRIBUTION TO THE GENETICS OF MILK GLAND ACTIVITY IN CATTLE

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IN a study of the composition of milk, based on comparisons between a number of twin pairs, it was found that the percentages of protein and of lactose *at fixed levels of percentages of fat* are chiefly genetically determined (BONNIER and HANSSON, 1946). That is to say: in two sets of samples of milk, all of which have equal percentages of fat (or, more correctly, have fat percentages the range of variation of which is only 0,1 %), the differences between the means of the two sets in protein percentage or in lactose percentage are within the limits of random variation if the two sets are taken from two identical twins, whereas, from a statistical point of view, the means of the two sets may be very widely apart if they are taken from two unrelated cows. The studies in question are based on 2245 samples taken from 29 pairs of twins of which 19 are identical, 7 are fraternal and 3 are uncertain as to the nature of the twinships. Table 1, in which some of the statistical figures are given, is an excerpt from Tables 3 and 4 of the article cited. The orders of magnitude of the P's are found by comparing the ratios of the mean squares in question by the error mean square.

In most of the cases the two twins of a pair had been treated or fed differently but, so far, it has not been possible to show any certain influence from this differential treatment on the differences between protein or lactose percentages at fixed percentages of fat.

*The number of genes in action.* — A more searching analysis (still always made by comparisons *within* groups of fat percentages, the grouping unit of which is 0,1 %) of the genetical relationships can, however, be made. The average mean squares, corresponding to the comparisons between the means of two identical twins, 0,0713 for protein and 0,1523 for lactose, may be said to constitute a measure of the average difference an animal would show if it did live two different lives. These mean squares have therefore been used as a common base for comparisons between other mean squares by dividing the latter by the former. Thus each mean square corresponding to the difference of the means of two twins of each separate twin pair has been divided by the above-

TABLE 1. *Analysis of variance of the differences between percentages of protein and of the differences between percentages of lactose at fixed fat percentages.*

	Degrees of freedom	Protein		Lactose	
		Mean square	P	Mean square	P
Between groups of different fat percentages.....	45	4,2830		0,7982	
At fixed fat percentages					
Between twin pair means (= between unrelated animals)	503	0,2288	$\leq 0,001$	0,4043	$\leq 0,001$
Cow versus twin sister					
identical twins ...	168	0,0713	$\geq 0,05$	0,1523	$\geq 0,05$
uncertain twins ...	45	0,0765	$\geq 0,05$	0,1882	Appr. = 0,05
fraternal twins ...	82	0,1048	0,01—0,001	0,1920	Appr. = 0,01
Within cows (error)	1401	0,0697		0,1359	
Total	2244				

mentioned figures. These ratios are given in Table 2. Furthermore, twin pairs for which this ratio is less than 1,5 for protein as well as for lactose, 18 in number (13 identical, 3 fraternal and 2 uncertain), have been selected, and the mean squares corresponding to the  $\frac{17 \times 18}{2} = 153$  differences between two such twin pair means have

been computed. As above, these 153 mean squares are divided by 0,0713 for protein and by 0,1523 for lactose and the ratios are collocated in two tables (Table 3 for protein and Table 4 for lactose). Finally, all ratios are classified in groups with a grouping unit of 0,5 and the distributions are computed for the 19 identical pairs, the 7 fraternal pairs and for the 153 twin pair combinations (Table 5, Fig. 1).

Before trying to interpret the tables it may be well to explain some of the figures. If we look at Table 3, we find, for instance, that the ratios are: for pairs 9—11, 1,387, for pairs 9—14, 1,472, thus two moderate ratios. But the ratio for pairs 11—14 is very much larger, 11,397. And there are other cases of a more or less similar kind. It may seem natural to expect that, if we have three pairs, and if the difference

TABLE 2. *Ratios between, on the one hand, the mean squares between the two twin means and, on the other, the average mean square between means of two identical twins (0,0713 for protein and 0,1523 for lactose).*

No. of twin pair		Nature of twinship	Ratio	
In the barn	For referen- ce to Tables 3 and 4		Protein	Lactose
4—5.....	1	Identical	0,738	1,344
8—9.....	2	»	0,400	0,405
106—107.....	3	»	0,328	0,374
115—116.....	4	»	0,180	0,147
119—120.....	5	»	0,829	1,175
121—122.....	6	»	1,021	0,609
205—206.....	7	»	0,389	0,681
207—208.....	8	»	0,586	0,541
209—210.....	9	»	1,293	0,415
213—214.....	10	»	0,878	1,268
725—726.....	11	»	1,282	0,899
818—819.....	12	»	0,839	0,936
821—822.....	13	Uncertain	0,797	1,229
823—824.....	14	Identical	1,397	0,693
736—737.....	15	Fraternal	0,881	0,721
807—808.....	16	»	1,499	0,823
830—831.....	17	»	1,224	1,324
716—717.....	18	Uncertain	1,449	0,545
123—124.....		Identical	2,756	1,464
201—202.....		»	1,659	1,020
203—204.....		»	0,958	1,883
215—216.....		»	0,610	2,558
921—922.....		»	0,853	1,536
117—118.....		»	1,793	1,389
906—907.....		Fraternal	0,637	1,456
915—916.....		»	2,450	1,229
757—758.....		»	2,006	1,312
647—648.....		»	1,060	2,104
15—16.....		Uncertain	0,929	1,978

between the first and the second as well as that between the second and the third is small, then also the difference between the first and the third ought to be small. There are two reasons for this not always being the case. Suppose first that all three pairs were represented only within one and the same group of fat percentages. If the numeric value of the difference between the first and the second pair is  $a$ , and





TABLE 5. *Grouping of the ratios from Table 2 (19 identical twin pairs and 7 fraternal twin pairs) and from Tables 3 and 4 (153 twin pair combinations = unrelated animals).*

Range of ratios	P r o t e i n						L a c t o s e					
	Number of ratios			In percent of totals			Number of ratios			In percent of totals		
	Iden- tical twins	Frater- nal twins	Unrelat- ed ani- mals	Iden- tical twins	Frater- nal twins	Unrelat- ed ani- mals	Iden- tical twins	Frater- nal twins	Unrelat- ed ani- mals	Iden- tical twins	Frater- nal twins	Unrelat- ed ani- mals
0.00—0.10 .....	4		2	21.0		1.3	4		4	21.0		2.6
0.50—0.99 .....	8	2	13	42.2	28.6	8.5	6	2	29	31.6	28.6	19.0
1.00—1.49 .....	4	3	19	21.0	42.8	12.1	6	4	36	31.6	57.1	23.5
1.50—1.99 .....	2		26	10.5		17.0	2		31	10.5		20.3
2.00—2.49 .....		2	21		28.6	13.7		1	16		14.3	10.5
2.50—2.99 .....	1		23	5.3		15.0	1		11	5.3		7.2
3.00—3.49 .....			12			7.8			7			4.6
3.50—3.99 .....			8			5.2			4			2.6
4.00—4.49 .....			6			3.9			1			0.7
4.50—4.99 .....			4			2.6			2			1.3
5.00—5.49 .....			4			2.6			1			0.7
5.50—5.99 .....			3			2.0			1			0.7
6.00—6.49 .....			2			1.3			1			0.7
6.50—6.99 .....			2			1.3			1			1.3
7.00—7.49 .....			3			2.0			2			2.0
7.50—7.99 .....									3			
8.00—8.49 .....									1			0.7
8.50—8.99 .....									1			0.7
9.00—9.49 .....												
9.50—9.99 .....			3			2.0						
10.00—10.49 .....						0.7						
10.50—10.99 .....			1			0.7						
11.00—11.49 .....			1			0.7			1			0.7
Total	19	7	153				19	7	153			

between the second and third is  $b$ , then the difference between the first and the third may be either  $a - b$  or  $a + b$ . Suppose the latter to be the case. The mean squares corresponding to the three differences are then  $\frac{a^2}{2}$ ,  $\frac{b^2}{2}$  and  $\frac{a^2 + b^2 + 2ab}{2}$ . This means that if two of the figures in Table 3 or 4 are  $a$  and  $\beta$  the third may be of the order of magnitude  $a + \beta + 2\sqrt{a\beta}$ . This explains several of the cases, but not all. And,

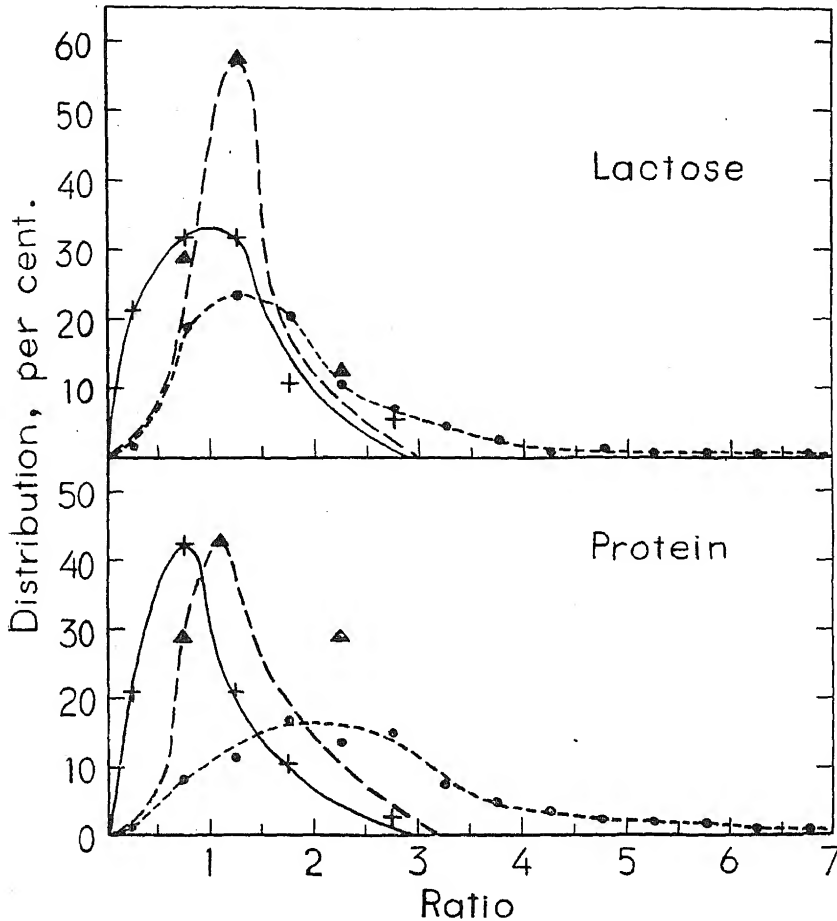


Fig. 1. Graphs corresponding to the distributions of the mean square ratios in Table 5. Crosses = comparisons between identical twins; triangles = comparisons between fraternal twins; dots = comparisons between unrelated animals (= between twin pair means). The curves are fitted by free-hand. (Therefore in the case of fraternal twins, with only 3 groups represented, see Table 5, the fitting is very haphazardous.) For lack of space, the curves for unrelated animals are broken at the ratio-value of 7.

for instance, not the example given, as we have  $1,387 + 1,472 + 2\sqrt{1,387 \times 1,472} = 4,288$ , which is very much less than 11,397. The other reason is random variation. When two pairs are compared, all groups of fat percentages in which both of the pairs are represented will automatically be included in the computation of the mean square of the pair means' difference. But if only one of the pairs (or none) is represented within a certain group of fat percentages, this group is automatically excluded. Thus, if the first and the second pairs are represented within the group of fat percentages 4,00—4,09 but not the third pair, this group contributes to the mean square corresponding to the comparison between the first and the second pair but not to the two remaining comparisons. Likewise, if the second and the third pairs are represented within the group of fat percentages 4,10—4,19 but not the first pair, this group will contribute to the mean square corresponding to the comparison between the second and the third pair but not to the two remaining comparisons. Thus, none of these two groups of fat percentages will contribute to the mean square corresponding to the comparison between the first and the third pair.

Looking now at the distribution of the ratios (Table 5, Fig. 1), it is evident that the range of their variation is smallest for the intra-identical twin comparisons, somewhat larger for the intra-fraternal twin comparisons, and very much larger for the comparisons of twin pair means (i. e. for the comparisons between unrelated animals). This corresponds of course to the data put together in Table 1. But it gives a more apparent and visual picture of the facts. The type of variation in the case of intra-identical twin comparisons must be due only to external (and random) causes. If only one single pair of genes was responsible for the differences between unrelated animals, there would be at most three different values round which the corresponding 153 ratios would vary. And, likewise, if only very few pairs of genes did act, some peaks ought to be found in the curves of distribution of the 153 ratios. But this does not appear to be the case, and thus it seems fair to conclude that several pairs of genes are in action. Hence we are probably concerned here with a case of polygenic effect.

It must be emphasized that the number of genes in action may be still greater, as it is possible that a certain gene difference which is active at one level of fat percentage may be inactive at another level. In such a case other gene differences must be active at that new level.

If the figures of Tables 3 and 4 are compared, it will be found that large ratios in one of the tables do not at all correspond to large ratios

in the other table. Grouping the ratios as in Table 5 with a grouping unit of 0.5 and computing the correlation of the 153 ratios for protein and for lactose, a correlation coefficient of  $-0.05$  is found. The signification of this is that different — or at least partly different — sets of genes are in action when the percentage of protein and the percentage of lactose are determined at a certain level of fat percentage.

### SUMMARY.

(1) It has been shown in an earlier article that in cow's milk the percentages of protein and of lactose at fixed levels of fat percentages are chiefly genetically determined.

(2) A comparison of the variation in the percentages of protein and of lactose at fixed levels of fat percentages between twin pair means (i. e. between unrelated animals) with the variation between the means of two identical twins has led to the conclusion that several genes must be in action.

(3) It is also shown that different sets of genes are responsible for the percentages of protein and for the percentages of lactose at fixed levels of fat percentages.

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# ON THE FORMATION OF BIVALENTS IN SOME PENTATOMIDS (HEMIPTERA)

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DURING a casual examination some ten years ago of preparations showing the spermatogenesis of *Dolycoris baccarum* L., a Hemipteron belonging to the family *Pentatomidae*, the stages of diakinesis particularly attracted my attention. Except in the latest stages of diakinesis no normal bivalents at all were visible, the homologous chromosomes being more or less separated from one another, so that a count of the separate chromosome elements gave the full diploid number of 14 characteristic of this species (12 autosomes, *X* and *Y*). In the late stages of diakinesis and the first metaphase, on the other hand, 6 normal autosome bivalents in addition to the *X* and *Y* chromosomes were visible. It was my intention at the time to embark on a closer investigation of the matter, but other work in progress and the war years then intervening obliged me to postpone the realisation of this intention for some time.

A few years ago, when SCHRADER's investigation on the formation of tetrads in another Pentatomid, *Rhytidolomia senilis* SAY, published as early as 1940 fell into my hands, I found that he had there elucidated the same phenomenon that I had noticed in *Dolycoris*. Since, however, the conditions appearing in *Rhytidolomia* did not, to my mind, correspond in every way with those I found in *Dolycoris*, I determined to carry out a corresponding investigation with respect to *Dolycoris* also.

*Material and methods.* — My material was collected in July, 1945, on the Hanko peninsula in the territory surrounding the zoological station of Tvärminne. In addition to *Dolycoris*, specimens of another, closely related Pentatomid, *Carpocoris purpureipennis* DE G., were also taken. All the specimens of which testes were fixed were in the last larval stage. In this stage all the phases of meiosis are plentifully represented. Spermatogonia, on the other hand, are but seldom found. In the collecting of the material I enjoyed the friendly assistance of Dr. HÅKAN LINDBERG, for which I should like here to express my best thanks.

The material was fixed in BENDA, CARNOY, RANDOLPH and BOUIN-

ALLEN. Of these, RANDOLPH gave the best results, at least as far as the early stages of meiosis were concerned. The microtome sections were made 10 or 15  $\mu$  thick. The preparations were stained with iron haematoxylin, crystal violet, or by the FEULGEN method. No smears were made.

The pictures were drawn with an ABBE drawing apparatus at the height of the work-table, using an immersion objective of 100 $\times$  and an ocular of 25 $\times$ . Since the drawings were reduced in size by one-half in making the printing-plates, the magnification is now about 2250 $\times$ .

*Observations.* — In *Dolycoris*, as in so many other Hemiptera, it is impossible to make any observations on the primary pairing of the chromosomes, since the earliest stages of meiosis, the leptotene and presumably also the zygotene stage, are characterized by particularly strong synizesis. The thin chromosome threads are drawn together into a single tangle in the centre of the nucleus (Fig. 1), a typical central synizesis. When the conditions causing synizesis have passed, the close tangle of chromosomes loosens and begins to expand in every direction, tending to fill evenly the whole nucleus. In this stage the chromosomes are comparatively thick, strongly stained threads (Fig. 2).

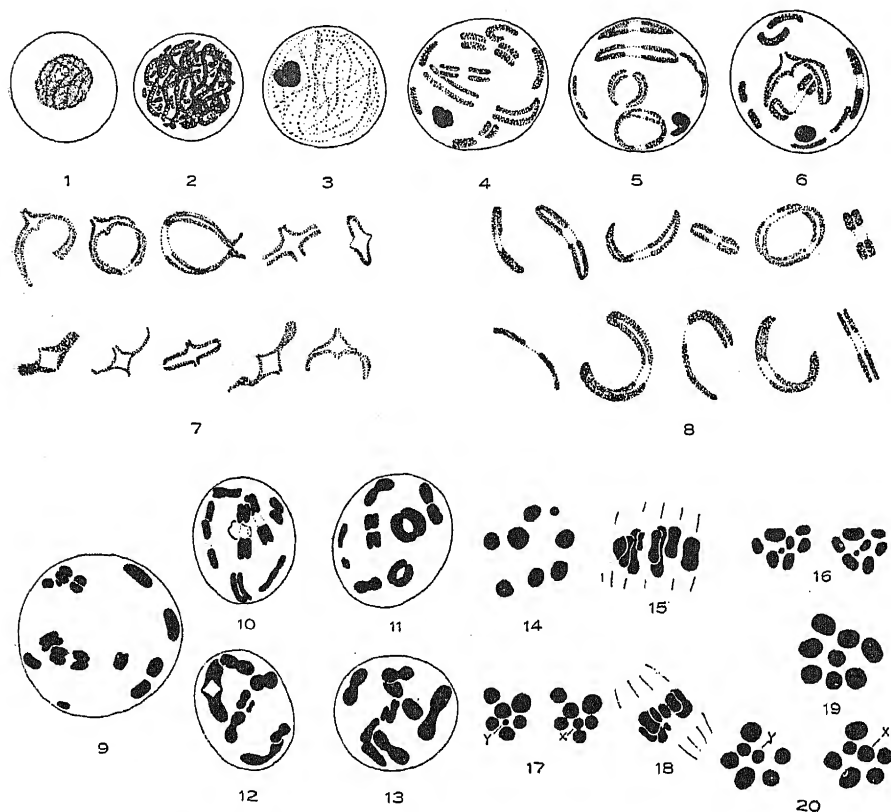
This pachytene stage does not, however, seem to be of long duration, the change to the diffuse stage taking place immediately all the signs of true synizesis have disappeared (Fig. 3). The chromosomes now lose their ability to stain almost completely, making it impossible to find out anything about their structure. This stage is also accompanied by a very appreciable increase in size of the nucleus. In contradistinction to the autosomes, which are entirely diffuse, the sex chromosomes appear in this stage as strongly stained, compact bodies. The *X* and *Y* chromosomes here show a strong tendency to associate (cf. GEITLER, 1939, p. 216). They are on this account generally visible either as a sort of doublet, in which the smaller *Y* appears just as if it were but a bulge on the side of the larger *X* (Figs. 3—5), or entirely fused together, leaving only a single, spherical and apparently homogeneous body (Fig. 6). The close packing of the chromosomes makes it impossible to decide whether the sex chromosomes are heteropycnotic already during the synizesis and early pachytene stages.

No nucleolus, or at least no discernible one, appears during the prophase of meiosis, for no other definite, compact body other than the *XY* complex is to be observed during these phases. Since the sex chromosome complex appears exactly the same in both FEULGEN and

crystal violet preparations, it cannot be accompanied by a nucleolus.

As it is impossible to ascertain anything about the pairing relations and chiasma formation of the chromosomes before or during the diffuse stage, the stage when the chromosomes again become visible after the diffuse stage is of particular importance for the investigation of bivalent formation.

Characterizing the stage in question in *Rhytidolomia*, SCHRADER (1940) mentions that the diploid number of chromosome elements



Figs. 1—18. *Dolycoris baccarum*. — Fig. 1, synizesis (leptotene stage). — Fig. 2, pachytene stage. — Fig. 3, diffuse stage. — Figs. 4—6, early diakinesis. — Fig. 7, bivalents with typical not terminalised chiasmata in early diakinesis. — Fig. 8, bivalents with »super-terminalised distance chiasmata» in early diakinesis. — Figs. 9—13, late diakinesis. — Fig. 14, first metaphase plate. — Fig. 15, first metaphase plate in side view. — Fig. 16, sister plates of first anaphase. — Fig. 17, second metaphase; the same plate in two different levels. — Fig. 18, second metaphase in side view. — Figs. 19 and 20, *Carpocoris purpureipennis*. — Fig. 19, first metaphase plate. — Fig. 20, second metaphase, the same plate in two different levels.

occurs and that the homologous chromosomes are entirely isolated from one another, there being no visible connections and even considerable intervals between them. Thus, he affirms (1940, p. 127) that the condition in question is one with no sign of »parasynaptic pairing» or chiasma formation.

The situation in *Dolycoris* is largely analogous (Figs. 4—6). There is very often the full diploid number of 12 autosome elements. Their relative position, however, is nearly always such as to make it easy to see which of them are paired together, even though definite morphological differences do not exist between all of the chromosomes. Very often, though not always, a very thin thread connects homologous chromosomes, which shows that we nevertheless have bivalents, notwithstanding the fact that the connection between their two components is extremely weak. The greater the distance between the homologues the weaker this connection thread generally is, and in extreme cases there appears, as stated above, to be no connection at all (Fig. 4). Inasmuch, however, as also in these cases the topographical relations between the chromosomes are such as to suggest some bond connecting the homologues, even though it is invisible, it seems to me very possible that such a bond actually always exists, though it can no longer take stain on account of its thinness.

In some cases, however, the homologues are not separated from one another but form typical end-to-end pairs. Finally, I have in some cases observed figures showing entirely clear and typical chiasmata (Figs. 6 and 7). Mostly these chiasmata are already very near complete terminalisation, though sometimes they still have a good distance to wander. Usually there is only one chiasma for each bivalent, but in the larger bivalents two chiasmata are also often found, terminalising on opposite ends. In rare cases one of the smaller bivalents may also form two chiasmata.

It is, thus, impossible to observe directly what occurs during the diffuse stage, comprising the latter part of the pachytene and most of the diplotene stage. But the fact that chiasmata are present at all, even though they are few, in the stages immediately following the diffuse stage gives indications that help to solve the question and throw a decisive light on the problem of bivalent formation in *Dolycoris*. For, in my opinion, it is quite apparent that chiasmata have formed normally and in the usual manner during the diffuse stage but have in the main had time to terminalise before becoming chromatinized sufficiently to be visible. Only a few have been so long delayed in terminalisation

that interstitial chiasmata may still be observed when the chromosome becomes visible.

The prophase of meiosis in *Dolycoris* is, then, characterized by rapid progress of chiasma terminalisation compared to the progress of chromatinisation and contraction of the chromosomes. When the chromosomes become visible after the confused stage, this latter development has reached a degree generally characterizing the diplotene stage, whereas terminalisation has in most cases progressed not only to the maximum degree, attained generally only in late diakinesis, but even beyond that, going over into a kind of »super-terminalisation» manifesting itself in a moving apart of the chromosome partners, often very far indeed from one another, the terminal connection between them stretching to a very thin thread.

The changes that the chromosomes thereafter pass through in the last stages of the meiotic prophase (Figs. 9—13), that is to say, up to the metaphase of the first maturation division, are founded on their continued chromatinisation and contraction, in addition to which the halves of each bivalent approach each other. In some cases the contraction and chromatinisation have attained almost their maximum degree even though the homologous chromosomes are still widely separated (Figs. 9 and 10), but generally they approach each other in the same measure as chromatinisation and contraction progress. Sometimes, it is true, thin connecting threads may be observed between the homologues that are approaching each other, like the threads that sometimes appear already in early diakinesis, but often there is no bond until the compact chromosomes have attained direct contact.

This final formation of bivalents generally takes place by an association of the homologous chromosomes at only one end. In the case of the large autosome pair, however, this association comparatively often takes place at both ends, forming a ring (Fig. 11). Thus, the large autosome appeared as a ring in 14 (or 14.9 %) of the 94 diakinesis nuclei examined. Smaller autosomes cannot be identified, but in 5 out of a total of 54 cells I was able to ascertain that one of the smaller autosome pairs formed a ring (Fig. 11).

The maturation divisions (Figs. 14—18) excite no particular comment, for they are in entire accord with corresponding stages in other Hemiptera. X and Y do not pair in the first division even though they generally lie side by side or at least quite close to one another in diakinesis, probably due to their close association in the early stages of meiosis. Thus, 8 separate chromosome elements are always visible in

the metaphase plate of the first division, which means that X and Y divide equationally in the first division. There is practically no interkinesis, the first division anaphase plates (Fig. 16) going over directly into the second maturation division. X and Y show a tendency to pair already during the first anaphase, and in the second maturation division (Figs. 17 and 18) they then move to different poles.

The circumstances at bivalent formation in *Carpocoris purpureipennis*, the other Pentatomid examined, correspond to the above in every way. It is also in other respects karyologically very similar to *Dolycoris* (Figs. 19 and 20). Thus, it has the same number of chromosomes, and the sex chromosomes behave in a corresponding manner. However, the Y chromosome is much larger in size than in *Dolycoris*, being about as large as the smallest of the autosomes (Fig. 20).

*Discussion.* — Though the process of bivalent formation in *Dolycoris* seems at first glance to depart radically from the normal, the facts presented above show that there is really no fundamental difference. The most important departure from normal lies in the exceptionally early terminalisation of the chiasmata, which, as already mentioned, appears in the main to take place already during the diffuse diplotene stage.

Besides being earlier, terminalisation is also more complete than usual. This fact confirms my statement earlier made in another connection (OKSALA, 1943, p. 21) to the effect that the earlier — relatively to contraction — terminalisation takes place the more completely it develops. It is but natural that it should be easier for the chiasmata to move when the chromatids composing the bivalent are still thin and weakly spiralled, and not burdened with a great amount of thymonucleic acid either. Thus terminalisation does, in fact, quite apparently take place in *Dolycoris* more easily than normal, for it attains its maximum degree very quickly and usually even exceeds it, for I interpret the moving apart of the bivalent halves in early diakinesis more as a sort of »super-terminalisation» than as true breakage of chiasmata and break-down of the bivalents. This conception of the matter may, in my opinion, be defended on the following grounds.

A visible though thin bond often persists between the halves of the bivalents all through diakinesis. This being the case, we are justified in saying that the chiasma still exists. And even when there is no visible bond the position of the homologous chromosomes relative to one another is such as to give a strong impression of their belonging together. As far as I can see, we may well consider an actual bond to

exist, even though too thin to stain. Also, the manner in which the bivalents attain their final shape in the last stages of diakinesis speaks for retention of the chiasmata. The attraction between homologous chromosomes at this stage is remarkable inasmuch as it generally unites the small autosomes at only one end, the largest autosome pair, however, either at one or at both ends. Herein there appears a clear analogy to chiasma formation, for in the smaller autosomes there is generally formed but one chiasma, terminalising on one end, whereas the largest autosome may also form two chiasmata, thus often appearing as a ring. If, now, this terminal attraction appearing in the last stage of diakinesis were a phenomenon entirely independent of previous chiasma formation, it would be incomprehensible why it should take place in the manner just described. Why should it not then unite all chromosomes at both ends or all at only one end? Actually, however, the original chiasmata are quite evidently renewed. *Thus we have reason to presume that the terminal chiasmata have at no stage completely disappeared, but much rather have super-terminalised and stretched exceedingly thin, yet been retained as effective »distance chiasmata» all throughout diakinesis.*

The phenomena here remarked in *Dolycoris* are, in my opinion, of a nature to throw new light in some respects also on the case of *Rhytidolomia* described by SCHRADER. This inasmuch as meiosis in these two species shows so much in common that it appears to me justifiable to interpret both cases on the same basis. Thus, *Dolycoris* may in respect to bivalent formation be considered as a sort of intermediate form between normal Hemiptera and *Rhytidolomia*. The tendency already clearly observable in *Dolycoris* has evidently attained its peak in *Rhytidolomia*. On these grounds it would seem to me to be not too daring to assume more or less normal chiasma formation to occur in *Rhytidolomia* also, even though this as well as the terminalisation of the chiasmata takes place entirely during the diffuse stage. In *Dolycoris*, on the other hand, the last vestiges of chiasmata are still perceptible when the chromosomes reappear clearly defined after the diffuse stage. An effective bond between homologous chromosomes throughout diakinesis quite apparently exists also in *Rhytidolomia*, to judge from SCHRADER's illustrations (the microtome sections only are to be taken into account in this connection), and the selective end-to-end attraction between these chromosomes apparent in the last stage of diakinesis would, in *Rhytidolomia* as in *Dolycoris*, be unexplainable were it independent of primary chiasma formation. The final formation

of bivalents at the close of diakinesis need, therefore, in no wise be considered the result of some entirely new »pairing effect«. On the contrary, it may simply be a question of the familiar phenomenon that on entering into the metaphase of the first maturation division the bivalent halves contract to the limit and cling to each other more closely.

To my mind *there is therefore no reason to consider Rhytidolomia as unique in respect to bivalent formation any more than Dolycoris and Carpocoris are.*

In conclusion I cannot desist from saying a few words concerning bivalent formation in another Hemipteron, *Alydus calcaratus* L. (*Coreidae*). REUTER, who has described the whole development here with great exactitude, remarks that there is nothing that would indicate segment exchange (nor, therefore, chiasma formation). The original, typical parallel pairing simply changes over into end-to-end pairing at a certain stage. Since the diffuse stage is not so marked, and inasmuch as all the phases of spermatogenesis are clearly to be observed, step by step, there should be no question of a veiled chiasma formation such as we found in *Dolycoris*. Still, it is not impossible that the bivalents arise on the basis of chiasma formation in *Alydus* also, if we consider the chiasmata as localized out to the extreme end of the chromosomes. Some of the illustrations shown by REUTER (e. g., Figs. 45 and 83) seem clearly to speak for such an interpretation.

### SUMMARY.

The investigation concerns bivalent formation during spermatogenesis in two members of the family of *Pentatomidae*, *Dolycoris baccarum* and *Carpocoris purpureipennis*.

In most cases terminalisation is completed during the so-called diffuse stage, and in consequence non-terminalised chiasmata are extremely seldom visible when the chromosomes become clearly defined in early diakinesis. The chromosomes composing each bivalent have at this point moved apart, often even very far from one another, so that the diploid number of chromosomes is often visible in the nucleus. Sometimes the bivalent halves are united by a thread stretched very thin, sometimes again no bond is visible, which may possibly be explained as due to these threads being too thin and fine to stain. In any case it is apparent that some effective connection exists between the bivalents halves. The true terminal chiasma has thus through

»super-terminalisation» changed into a »distance chiasma». At the end of diakinesis the homologous chromosomes finally move into close contact, and the bivalent attains its final form.

In the opinion of the author, the formation of bivalents in *Rhytidolomia senilis*, investigated by SCHRADER, must also be interpreted on the same principles, i. e. assuming the chiasma to persist all through diakinesis. Thus all of these Pentatomids differ from the normal only in earlier and more complete terminalisation of chiasmata in time relation to chromosome contraction and chromatinisation during the prophase of meiosis.

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# TOTALE INVENTIERUNG DER MIKRO-TYPEN EINES MINIMIAREALS VON TARAXACUM OFFICINALE

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(With a Summary in English)

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**D**IE grosse Variabilität der Gattung *Hieracium* ist lange bekannt. Schon DARWIN nannte sie eine protäische Gattung. NÄGELI und PETER haben grosse Mühe auf eine Aufklärung der mitteleuropäischen Formenkreise verwendet und DAHLSTEDT und viele andere nordische Forscher bezüglich der nordischen. Gewisse nordische Hieracien (vor allem *Archieracia*) sind hierbei ganz ausserordentlich aufgespaltet worden. Man rechnet schon mit mehr als 2.000 »Mikrospezies«. Andere Formenkreise hat man nur teilweise oder gar nicht einer derartigen »Pulverisierung« unterwerfen können. Die Ursache ist nunmehr klar. Es ist hierfür der Grad der agamospermen Samenbildung entscheidend. Nur bei totaler Agamospermie ist eine Unterscheidung bestimmter Formentypen möglich, bei partieller ist dies kaum und bei sexueller Befruchtung gar nicht durchführbar.

Zu Beginn dieses Jahrhunderts wurde durch DAHLSTEDT die Polymorphie von *Taraxacum officinale* entdeckt. Diese Population hat man bis 1900 als eine einzige Spezies betrachtet und innerhalb dieser sind nur einige wenige Varietäten unterschieden worden, die hauptsächlich auf extreme Standorte begrenzt waren. LINNÉ führte *Taraxacum* nicht einmal als eigene Gattung auf. *T. officinale* wurde von ihm der Gattung *Leontodon* zugerechnet und *Leontodon Taraxacum* genannt.

Während den letzten Dezennien haben mehrere nordische Forscher, ausser DAHLSTEDT vor allem LINDBERG FIL., MARKLUND und HAGLUND, eine gründliche Untersuchung der *Taraxaca* des Nordens vorgenommen. Die Anzahl der von ihnen aufgestellten Arten beträgt nach einer Zusammenstellung von HYLANDER (1941) nicht weniger als 450. Natürlich entsteht bei dieser Artenfülle sogleich die Frage: Hat man damit die ganze Anzahl der unterscheidbaren Typen erreicht? Und weiter: Können diese Arten überhaupt auseinander gehalten werden? Gibt es zwischen ihnen nicht noch weitere Formen, die ihre Unterscheidung

erschweren oder unmöglich machen? Ist die Anzahl der erblichen Formen, der Biotypen, nicht so gross, dass es ganz aussichtslos wäre, eine Aufteilung in Kleinarten, Mikrospezies, zu versuchen?

Die nordische Population der *Taraxacum officinale* ist für eine Untersuchung des Formenreichtums jedenfalls von einem Gesichtspunkt aus besonders geeignet; sie bildet nämlich stets Samen ohne Befruchtung, zeigt also *vollkommene* Agamospermie. Unter den Formen vom nördlichsten Skandinavien bis nach Dänemark hat man keine einzige sexuelle oder partiell sexuelle Form gefunden. (Ein einziger bei Gothenburg angetroffener Typus, *T. obtusilobum*, ist aller Wahrscheinlichkeit nach eingeführt.) Die Konstanz der Typen ist deshalb eine *vollständige*. Eine *Neuerzeugung* von Formen durch Kreuzung und Spaltung erscheint daher *ausgeschlossen*. Das ist ein wichtiger Ausgangspunkt.

Nun fragt man also: Ist die Variabilität überhaupt erschöpfbar? Kann die Anzahl der Biotypen, obgleich sehr gross, durch das hoch entwickelte Unterscheidungsvermögen des Spezialisten wirklich festgestellt werden?

Das ist eine Frage, die taxonomisch kaum zu beantworten ist. Denn der Systematiker unterscheidet ja immer Durchschnittstypen. Unter einer bestimmten Bezeichnung, Beschreibung, fasst er *Ähnliches* zusammen. Ob die Differenzen gross oder klein sind, ob er mit Arten oder Formen arbeitet, immer ist sein Auge das Werkzeug und ein Durchschnittstypus das Mass. Das schon Unterschiedene *sucht er auf* und findet hierbei auch Neues. Ob aber kleinere Abweichungen als die durch die »Kleinarten« bestimmten da sind, dafür hat er kein Interesse; ganz natürlich deshalb, weil er dies nicht entscheiden kann.

Für die Entscheidung, ob die unterschiedenen Typen von *Taraxacum* nur kleinere Durchschnittstypen sind um die noch eine geringere Variabilität zu finden ist, oder ob sie eine reduzierte Anzahl von Biotypen darstellen, zwischen denen es keine Zwischenformen gibt, muss das Experiment herangezogen werden.

Man hat auch Kulturversuche vorgenommen. Hierbei haben sich die Formen als ganz konstant erwiesen. Bei unsrer heutigen Kenntnis ihrer Fortpflanzungsweise ist das ja selbstverständlich. Sie sind apomiktisch konstant durch ihre agamosperme Samenvermehrung, gerade so wie Pflanzen, die durch Bulbillen oder Stecklinge vermehrt werden, konstant sind. Da man ferner die *in der Natur als verschieden konstatierten* Formen in der Kultur geprüft hat, hat man über die *Totalvariabilität* keinen Aufschluss erhalten.

Um die Frage nach der totalen Variabilität, dem Biotypeninhalt

von *Taraxacum*, beantworten zu können, ist eine ganz andere experimentelle Anordnung erforderlich, bei der die zu untersuchenden Individuen *nicht ausgelesen*, sondern bei der *alle Individuen* eines Areals in den vergleichenden Kulturversuch gelangen.

Ich ging in folgender Weise vor. An Standorten mit einer reichen *Taraxacum*-Flora beutelte ich mit Tülltüten die Köpfchen *aller* Pflanzen ohne Ausnahme ein, *ob sie nun verschieden erschienen oder nicht*. Die Tülltüten sollten nur die Samen bei der Reife zurückhalten. Dicht schliessende Pergamintüten waren nicht notwendig, da keine gegenseitige Befruchtung stattfindet.

Zwecks Einsammlung wurden drei verschiedene Standorte gewählt. Von einem Grasrand zwischen den Bäumen einer Allee in Alnarp nahe Lund, der mehr als 50 Jahre unberührt geblieben war, wurden sämtliche Pflanzen einer Fläche von 5 qm eingebeutelt. Hier hatten die *Taraxaca* offenbar eine starke Konkurrenz mit den übrigen Pflanzen des Bestandes, hauptsächlich Gräsern, auszustehen. Überdies war der Bestand jährlich zweimal gemäht worden.

Nur 50 m von diesem Standort entfernt wurde ein weiterer von 5 qm für die Versuche gewählt. Er lag an einem Wegrand, ganz offen. Ein Bestand von Gramineen befand sich in Entwicklung, war aber noch nicht ganz geschlossen. Die Konkurrenz der *Taraxaca* war hier sehr viel geringer als im vorigen Bestand.

Fast 1 km von diesem Standort entfernt befand sich an einem Weg, der von einer Hecke gegen den Park von Alnarp abgegrenzt ist, eine sehr reiche *Taraxacum*-Flora. Der Standort lag sonnenoffen. Der Wegrand wird von Unkraut frei gehalten, weshalb die blühenden Pflanzen sich dort im vergangenen Herbst angesiedelt haben müssen. Offenbar stammten sie nicht nur von Pflanzen, die voriges Jahr dort gewachsen sind, sondern auch von Samen in der Gegend wachsender Pflanzen, die an der Hecke am Weiterfliegen gehindert worden sind und sich am Heckenrand angesammelt hatten. Die Pflanzen müssen hier deshalb zum grössten Teil als hemerophile Ansiedler betrachtet werden. Daher war an diesem Standort auch der grösste Formenreichtum zu erwarten. Da dies für die Untersuchung bedeutungsvoll war, wurden die Pflanzen eines grösseren Areals als an den obengenannten Stellen, nämlich von 40 qm, eingebeutelt. Insgesamt wurden also die Samen sämtlicher Pflanzen eines Gesamtareals von 50 qm für die weitere Untersuchung eingesammelt.

Im Vorsommer desselben Jahres (1934) wurden die Samenproben, 177 an der Zahl, in kleine Kästen ausgesät. Die Erde wurde nicht

sterilisiert, aber einer Tiefe entnommen, in der während des letzten Jahrzehntes keine Umgrabung stattgefunden hatte. Wenn hier überhaupt *Taraxacum*-Samen vorkamen, so konnten sie das Experiment nicht stören, da sie ihre Keimfähigkeit schon nach 4—5 Jahren verlieren. Es konnten auch während der ganzen folgenden Versuchszeit (bis 1940) keine Einmischungen festgestellt werden.

Früh im Herbst (August) wurden die Pflanzen im Botanischen Garten zu Lund auf Freiland ausgepflanzt. Jede Nummer, also jede Nachkommenschaft, bildete eine Parzelle mit 15 Pflanzen. Die Parzellen lagen ganz und gar in der bei dem Einsammeln benutzten Reihenfolge. Jeder Versuch einer Vereinigung ähnlicher Ausgangspflanzen oder ähnlicher Nachkommen in den Kästen wurde vermieden. Erst nachdem die Nachkommen auf dem Felde standen, nebeneinander und übersichtlich, wurde die *Beurteilung der Variabilität* vorgenommen.

Mit dieser vollständigen Inventierung eines Minimiareals habe ich also eine Methode benutzt, durch die die Variabilität voll und ganz beibehalten worden ist. Durch die Parzellen wird jede Ausgangspflanze multipliziert und alle Nachkommen werden auf einen begrenzten Raum vereint, sodass eine vergleichende Übersicht erhalten wird. Erst hierdurch wird es möglich, auch kleinere Differenzen festzustellen.

Schon im Rosettenstadium fiel es auf, dass die Parzellen keine kontinuierliche Variabilitätsreihe bildeten. Gegenüber einem ähnlichen Versuch mit Kulturpflanzen, mit dessen Verhalten ich durch meine frühere Züchtungsarbeit sehr vertraut war, war sogleich ein ganz bestimmter Unterschied zu erkennen. Hat man eine Parzellenreihe von Nachkommenschaften einzelner Pflanzen, so bilden diese eine fast kontinuierliche Serie, binnen der die Varianten keineswegs in Gruppen vereint werden können. Sogar bei selbstbefruchtenden Arten, wie z. B. mit Hafer, erhält man bei der Züchtung einer Landsorte dieses Resultat. Dies beruht natürlich darauf, dass es noch so viele Biotypen gibt, dass zwischen ihnen keine Lücken erkennbar sind. Das Auge hat für ein Unterscheiden keine Anhaltspunkte. Alles fließt. Wählt man zu einer derartigen Auslese einen Fremdbefruchter, wie z. B. Roggen, so wird die Variabilität natürlich noch kontinuierlicher.

Für *Taraxacum* war es, als die Rosetten im Spätherbst vollausgebildet waren, nicht schwierig, die Parzellen in bestimmte Gruppen zu vereinigen. Noch deutlicher traten die Unterschiede im folgenden Jahr, nachdem die Blüte eingetreten war, zutage. Innerhalb der Parzellen waren alle Pflanzen hochgradig übereinstimmend. Zu einer bestimmten Gruppe konnte ich in einigen Fällen viele Parzellen, die ganz

übereinstimmend erschienen, vereinigen, in anderen Fällen nur wenige, und schliesslich bildete in gewissen Fällen eine einzige Parzelle eine Gruppe. In dieser Weise erhielt ich 24 Gruppen. Von diesen waren indessen 6 anderen Gruppen sehr ähnlich und wichen nur in einzelnen Eigenschaften von diesen ab. Eine Parzelle war z. B. so auffallend später als der Typus, dem sie sonst anzugehören schien, dass die Differenz zwei Wochen betrug.

Es galt nun festzustellen, ob die letztgenannten Abweicher wirklich erbliche Kleinformen oder nur reine Modifikationen darstellten. Für die erste Alternative schien die Tatsache zu sprechen, dass alle Individuen der abweichenden Parzelle in derselben Richtung variierten.

Auch dieses Jahr wurden von allen Parzellen Pflanzen eingebeutelt, sodass Samen für weitere Versuche erhalten wurden und der ganze Versuch demnach wiederholt werden konnte. Beim Auspflanzen der neuen Parzellen im Herbst 1935 wurden die Gruppen übereinstimmender Parzellen vereinigt, sodass diese unter sehr ähnlichen Substratbedingungen wuchsen und so genauer miteinander verglichen werden konnten.

Schon dieses Jahr erwiesen sich von den erwähnten 6 Gruppen 4 als nicht oder nicht sicher abweichend. Die erwähnte sehr spätblühende Parzelle hatte dieses Jahr ganz dieselbe Blütezeit wie die Parzellen seines Typus. Offenbar war ihre Abweichung von rein modifikativer Natur. Überhaupt scheint die modifikative Variabilität bei *Taraxacum* eine ganz beträchtliche zu sein ohne aber die Grenzen zwischen den Typen unter gleichartigen Kulturverhältnissen zu verwischen.

Zwei der 6 Gruppen schienen dagegen untereinander und von den während des vorigen Jahres unterschiedenen deutlich abweichend zu sein.

Der ganze Versuch wurde 1936 noch einmal durch Samenanbau wiederholt, weil ich eine ganz sichere Beurteilung der Gesamtvariabilität erhalten wollte. Das Resultat war mit dem des vorigen Jahres vollkommen übereinstimmend. Die Parzellen konnten ganz scharf in 20 (18 + 2) Gruppen aufgeteilt werden.

Im Jahre 1938 fand nur eine teilweise Wiederholung statt. Die oben erwähnten 4 wahrscheinlich modifikativen Gruppen, die in bezug auf Pigmentierung, Rosettenfarbe, Zerschlitzung des Blattrandes oder Form und Richtung der Hüllblätter von ihren Gruppen abzuweichen schienen, wurden durch zwei Wiederholungen sehr sorgfältig geprüft, da es wichtig war festzustellen, ob ihre Abweichung, die ganz kontinuierlich erschien, von Jahr zu Jahr und in den verschiedenen Wiederholungen ein und desselben Jahres in dieselbe Richtung gehen würde.

Auch einige Parzellen, die während den früheren Jahren eine diminutive Abweichung von ihren Gruppen gezeigt hatten, wurden in derselben Weise weiter geprüft.

Auch 1939 wurde ein Teilversuch in gleicher Umfang und gleicher Anordnung wie 1938 ausgeführt.

Das Schlussresultat der ganzen, während 5 Generationen fortgesetzten Untersuchung war, dass sämtliche Nachkommen der Ursprungspflanzen sich auf 20 verschiedene Typen verteilten. Die anfangs als kleinere Varianten vermuteten Abweicher erwiesen sich *alle* als nicht erblich, waren demnach reine Modifikationen. Sie schwankten während den Versuchsjahren zwischen den Plus- und Minusgrenzen der Modifikabilität eines bestimmten Typus.

Die Unterschiede zwischen den Typen (»Mikrospezies«) waren mehr oder weniger ausgeprägt, *immer* aber deutlich, distinkt. Seitdem ich die Nachkommen der Ausgangspflanzen in Parzellen nebeneinander hatte, war ich in bezug auf ihre Aufteilung in eine Anzahl ganz bestimmter und definitiver Gruppen ganz im klaren. Es gab 20 Gruppen, zwanzig erblich verschiedene Typen.

Ich nannte diese Typen schlechtweg Nr. 1—20. Sie unter den schon beschriebenen 450 »Kleinspezies« aufzusuchen und mit gewissen dieser zu identifizieren wäre gewiss eine sehr mühsame Arbeit gewesen.

Es war für mich daher von grossem Vorteil, dass meine Typen von dem bekannten Taraxakologen GUSTAF HAGLUND durchgesehen und identifiziert wurden. Ich benutze die Gelegenheit ihm auch an dieser Stelle hierfür meinen herzlichen Dank zu sagen.

Folgende Typen wurden in meinen Versuchen festgestellt (auch die Anzahl der Parzellen jedes Typus, also seine Gesamtfrequenz an dem Minimiareal in der Natur wird angegeben):

<i>pynolobum</i> DT. ....	51	<i>aequilobum</i> DT. ....	1
<i>sagittatum</i> DT. ....	29	<i>Dahlstedtii</i> LINDB. FIL. ....	1
<i>retroflexum</i> LINDB. FIL. ....	26	<i>decurtatum</i> nov. ....	1
<i>polyodon</i> DT. ....	14	<i>dilatatum</i> LINDB. FIL. ....	1
<i>brevisectum</i> PALMGR. ....	13	<i>duplidens</i> LINDB. FIL. ....	1
<i>angustisquameum</i> DT. ....	11	<i>fulvum</i> RAUNK. ....	1
<i>caudatulum</i> DT. ....	7	<i>laeticolor</i> DT. ....	1
<i>cordatum</i> PALMGR. ....	7	<i>longisquameum</i> LINDB. FIL. ....	1
<i>sublaeticolor</i> DT. ....	4	<i>laeticeps</i> HAGL. ....	1
<i>mimulum</i> DT. ....	4		
<i>xanthostigma</i> LINDB. FIL. ...	2		
			Summe 177

Alle Pflanzen ausser einer einzigen konnten mit früher beschriebenen »Mikrospezies« identifiziert werden. Diese einzige ist die *decurtatum* nov. genannte. Sie stand dem *pycnolobum*-Typus nahe, wich aber in mehreren Eigenschaften, die sich durch alle der 5 Generationen schön wiederholten, ab. Schon die junge Rosette wich von *pycnolobum* durch ihre dem Boden angedrückten dunkelgrünen Blätter ab. Die *pycnolobum*-Rosette hat etwas aufgerichtete Rosettenblätter und die Blattfarbe ist graulich oder fast gelblich grün, ganz hell. Die Blattfarbe von *decurtatum* ist fast dunkel wie die von *tenebricans*, schwarzgrün. Die ausgewachsenen Rosetten haben kürzere Blätter als die der *pycnolobum*, weshalb sie kleiner erscheinen; auch sind sie stärker zerschlitzt. Diese Unterschiede gehen sehr gut aus den Fig. 1 und 2 hervor. Diese heben auch einen anderen, sehr charakteristischen Unterschied der Typen hervor, nämlich die kürzeren Stengel von *decurtatum*. Die äusseren Hüllblätter sind die festen, steif und kragenförmig abstehenden von *pycnolobum*, aber sie sind kürzer und der Kragen ist dichter. Durch die kurzen Stengel erinnert *decurtatum* an den *laeticolor*-Typus, bei dem sie jedoch so verkürzt sind, dass die Blütenköpfe von den Blättern fast überwachsen werden.

Dieser Typus erinnert wohl in bezug auf die Hüllblätter an *pycnolobum*, ist aber habituell ebenso stark von diesem abweichend wie z. B. *laeticolor*, *sublaeticolor* oder *laeticeps*. Der letztere Typus ist übrigens ausser in meinen Kulturen nur ein paarmal in der nächsten Umgebung von Lund aufgefunden worden (HAGLUND, 1934).

Unter den Typen gibt es also sowohl häufigere als auch seltenere. Aber der Unterschied ist in beiden Fällen ganz ausgeprägt. Unter den 51 Parzellen (Ausgangspflanzen) von *pycnolobum* oder den 29 von *sagittatum* waren ebensowenig feststellbare Unterschiede zu entdecken wie unter den 4 von *cordatum* oder den 2 von *xanthostigma*. Transgressionen traten nicht auf.

Die Sonderprägung der Typen war dagegen sehr verschieden und damit folgte die Leichtigkeit ihrer Unterscheidung. Die Typen *longisquameum* und *xanthostigma* erschienen mir so ähnlich, dass ich sie anfangs in eine Gruppe vereinigte. Erst als die Parzellen nebeneinander standen, traten auch ihre Unterschiede zutage. In ähnlicher Weise verhielten sich *brevisectum* und *mimulum*.

Überhaupt war eine Entscheidung der Frage, was ähnlich oder sehr abweichend war, zu grossem Teil davon abhängig, welche Merkmale für die Beurteilung ausgewählt wurden. Der *dilatatum*-Typus erschien mir, nachdem ich mein Material drei Generationen hindurch beobachtet

hatte, sowohl dem *longisquameum*- als dem *xanthostigma*-Typus sehr ähnlich. Sie schienen eine ganz intime Dreigruppe zu bilden. Betrachtete man aber die Sondermerkmale von *dilatatum*, so erinnerte der Typus durch seine stark zurückgeschlagenen Hüllblätter an *retroflexum*, in bezug auf gewisse Rosettenmerkmale an *cordatum* sowie in



Fig. 1. Der Mikrotypus *pynolobum*.

bezug auf die Blütenfarbe und die weitgeöffneten und lange offen stehenden Blütenköpfe an *pynolobum*.

Das angeführte Beispiel von *dilatatum* könnte man für jeden Typus vervielfältigen. Sämtliche Typen bildeten offenbar eine einzige Kombinationsgruppe, ein Kombinationsfeld, keine Serie oder Reihe, die in Sondergruppen aufgeteilt werden konnte.

Dass von *Taraxacum officinale* auf einer ausgewählten Fläche eine

*bestimmte und begrenzte Anzahl morphologisch distinkter Typen auftreten, die nicht durch kontinuierliche Zwischenformen verbunden sind, ist also durch meine Versuche festgestellt.*

Nun erscheint indessen die Frage berechtigt, ob nicht kleinere erbliche Formen vorkommen, nämlich solche, die *nicht morphologisch*



Fig. 2. Der Mikrotypus *decurtatum*. Die mit *pycnolobum* verglichen kleineren Rosetten und kürzeren Stengel treten deutlich zutage.

festgestellt werden können. Vielleicht gibt es physiologische Unterschiede, die die Assimilationsintensität, die Nährstoffsselektion, die Atmungskapazität usw. betreffen. In diesem Fall versagt das morphologische Experiment, mag es noch so sorgfältig durchgeführt sein, gänzlich. Der Einwand, dass es binnen den morphologischen Grundtypen kleinere Unterschiede geben könnten, wäre demnach nicht entkräftigt.

Dieser Einwand ist natürlich *theoretisch* berechtigt, aber kaum, wie

wir weiter sehen werden, real. Wir wissen nämlich nunmehr, dass die Wirkungsweise eines Gens pleiotrop ist. Ein Gen ist kein Organbildner, der eine einzige Eigenschaft bedingt, sondern das Gen schlägt im ganzen Organismus durch, und das nicht nur morphologisch, sondern auch biologisch und physiologisch. Das ist schon ein genischer Truismus.

Bei *Taraxacum* findet man, wenn eine Beobachtung der biologisch-physiologischen Eigenschaften der Typen vorgenommen wird, bald ganz ausgeprägte Unterschiede auch in dieser Hinsicht. Dies sei an einigen charakteristischen Beispielen veranschaulicht.

Gewisse Typen sind morphologisch dadurch ausgezeichnet, dass ihre Blattspreite nicht einheitlich grün ist, sondern einen braunen Blattsaum hat (*longisquameum*, *sublaeticolor*, *laeticeps*). Gleichzeitig zeigen sie stark verlängerte und helle Blattzipfel, helle Rosetten mit dem Boden stark anliegenden Blättern, in die Rosette tief versenkte junge Köpfchen und früheres Blühen im Frühjahr als die Mehrzahl der übrigen Typen. Mit dem Gen für das auffallendste morphologische Merkmal, das Makulieren, folgen also mehrere Eigenschaften der Struktur und Rhythmik der Pflanze.

Die Unterschiede in bezug auf das Erreichen der Blühfähigkeit während des ersten Entwicklungsjahres waren auch sehr ausgeprägt. Im Jahre 1934 wurden die Ende Mai geernteten Samen unmittelbar gesät und die jungen Pflanzen Anfang August ausgepflanzt. Ende Oktober blühten sämtliche Parzellen von *pynolobum*, welcher Typus die grösste Anzahl von Nachkommen, nämlich 51, hatte. Von *sagittatum*, von dem ich die nächsthöchste Parzellenzahl hatte, nämlich 29, war bei keiner einzigen Nachkommenschaft eine blühende Pflanze zu sehen. Vereinzelt Blühen zeigten *duplidens*, *polyodon* und *retroflexum*. Es ist also offenbar, dass gewisse physiologische Eigenschaften ebenso fest mit dem Typus verknüpft sind wie die morphologischen. Denn keine einzige *pynolobum*-, wie auch keine einzige *sagittatum*-Nachkommenschaft, wich von ihrem Typus ab.

In bezug auf den Nyktitropismus der Blüten waren bei den verschiedenen Typen auch ganz deutliche Unterschiede festzustellen. Die Köpfchen sind bei ihrem »Öffnen« und »Schliessen« sehr von der Sonne abhängig. Diese Eigenschaft kann daher nur an Tagen mit voller Sonnenbestrahlung beurteilt werden. Eine Untersuchung, die unter optimalen Bedingungen vorgenommen wurde, zeigte, dass bei der Mehrzahl der Typen schon früh Nachmittags ein Schliessen der Köpfchen eintrat. Für *sagittatum* war diese Zeit schon ein wenig ausge-

dehnt, bis 2 Uhr, für *dilatatum* ganz markiert, bis 3 Uhr, für *pynolobum* endlich sehr auffallend, bis 4 Uhr. Von besonderem Interesse ist, dass *pynolobum*, welcher Typus in bezug auf die Blühperiode eine Verkürzung zeigt, in bezug auf die Periode des Nyktitropismus eine Verlängerung aufweist. Die ganze Rhythmik dieses Typus ist demnach sehr abweichend, wahrscheinlich bedingt durch ein Gen mit vielseitig physiologischem Effekt. Dass ein und dasselbe Gen diese verschiedenen und extremen Eigenschaften bedingt ist wahrscheinlich. Sie bezeichnen wohl nur zwei während der ontogenetischen Entfaltung der Pflanze aufeinander folgende Reaktionen desselben Agenz (Genwirkung). Und vielleicht ist dieses Gen dasselbe, das auch für viele der extremen morphologischen Merkmale des Typus verantwortlich ist.

Ein ganz und gar sonderartiger nyktitropischer Typus ist *laeticeps*. Während sämtliche andere Typen bei bewölktem Himmel ihre Köpfchen schliessen oder geschlossen halten, hat *laeticeps* auch unter diesen Verhältnissen weit geöffnete Köpfchen. Der Typus nimmt hierdurch eine ganz auffallende Sonderstellung im *Taraxacum*-Versuch ein.

Die physiologischen Eigenschaften der Typen zeigen eine ebenso grosse Übereinstimmung wie die morphologischen. Alle Linien eines Typus reagieren in bezug auf Tropismen und Periodizitäten offenbar ganz gleichartig. Wäre die Variabilität biologischer und physiologischer Eigenschaften grösser als die der taxonomischen und habituellen, also der morphologischen, so wäre auch zu erwarten, dass die Reaktionsweise in bezug auf die Lebensäusserungen der verschiedenen Linien eines Typus in einigen Fällen Unterschiede aufweisen sollten. Das traf nicht zu. Die physiologischen Merkmale sind daher, soweit die Untersuchung diesbezüglich durchgeführt worden ist, von ganz denselben Genen abhängig wie die morphologischen. Der Effekt dieser Gene ist nur stark pleiotrop.

Dieses Ergebnis macht es auch sehr wahrscheinlich, dass kleinere biologisch-physiologische Unterschiede, ebensowenig wie taxonomische, innerhalb der Typen vorhanden sind. Diese sind ganz gleichförmig. Wie sind sie dann vom genischen und variationsbiologischen Gesichtspunkt aufzufassen? Dies ergibt sich dann auch unmittelbar. Es ist nur eine Deutung möglich: Sie sind *die kleinsten erblichen Varianten* der Population *Taraxacum officinale*. Sie sind die *Genotypen* oder, falls man diesen ganz identischen Terminus bevorzugt, *sie sind die Biotypen*.

Es ist also höchst wahrscheinlich, dass die »Arten«, die »Mikrospezies«, die »Typen« — wie ich sie bis jetzt schlechthin genannt habe

— die von den Taxonomen bei *T. officinale* ausgeschieden und beschrieben worden sind, nur die Rekombinanten der Population darstellen, also die Biotypen. Ganz sicher kann man indessen diesbezüglich nicht sein. Der Biotypus ist ja ein *genischer* Begriff, der *absolute* Identität der Konstitution bedeutet. Es ist deshalb schwierig, ihn für systematische Zwecke zu verwenden. Denn auch wenn die Varianten, die »Mikrospezies«, Biotypen sind, wie ich oben für *Taraxacum* wahrscheinlich machen konnte, so kann man es *nicht mit vollkommener Sicherheit* behaupten. Es besteht jedenfalls die *Möglichkeit*, dass zu ein und demselben Typus in seltenen Fällen mehr als ein Biotypus gehören kann, wenngleich die Unterschiede zu gering sind, um morphologisch oder physiologisch festgestellt werden zu können. Es ist unwahrscheinlich, aber möglich.

Es erscheint mir daher unrichtig, den Ausdruck Biotypus für systematische Einheiten zu verwenden, auch wenn man mit grösster Wahrscheinlichkeit annehmen kann, dass solche unterscheidbar sind. Aber ebenso unrichtig, ja widersinnig wäre es, eine Bezeichnung, die auf »Art« oder »Spezies« hinzielt, zu benutzen. Spezies bedeutet ja in der Systematik eine Population, einen Variationskreis, eine Kombinationssphäre, oft unzähliger Varianten. Eine »Mikrospezies« von *Taraxacum* ist ein *invariabler* Typus, ganz starr, eine *Kombination* innerhalb einer grösseren Population. Das zeigen die »Mikrospezies«. Sie können nicht in Kleingruppen oder Reihen zusammengefasst werden, da ihre Gesamtheit eine Population bildet, und sie selbst stellen nur die verschiedenen Kombinationen gewisser Grundgene dar.

Aber sie sind konstant, sagt man; sie behalten ihre Eigenschaften auch in Kulturversuchen unverändert bei. Natürlich, aber nur weil sie agamosperm sind. Sie würden aber, falls Samenvermehrung eintreten könnte, keine Konstanz aufweisen. Denn die Mehrzahl der Typen sind laut den Untersuchungen von GUSTAFSSON (1932) triploid. In der Nachkommenschaft *muss* dann Variabilität, und zwar eine sehr reiche und auffällige, eintreten. Das zeigen ja die fast unzähligen während den letzten Jahrzehnten ausgeführten Untersuchungen von Triploiden verschiedener Gattungen zu voller Evidenz. Da aber keine Befruchtung stattfindet, sondern die Vermehrung ganz vegetativ vor sich geht, wird ja die Reproduktion rein klonal, ganz als ob sie z. B. durch abfallende Bulbillen verursacht würde. Die »Konstanz« der Typen kann daher als Begründung für eine Speziesbezeichnung gar nicht herangezogen werden. Denn solchenfalls müssten alle Klonen des Pflanzenreiches als

Spezies aufgefasst werden. Und das ist eine unheimliche Konsequenz, vor der *jeder* Systematiker zurückschrecken würde.

Da man im abgehandelten Fall keinesfalls die Bezeichnung Spezies und nicht gern den Ausdruck Biotypus für die Varianten verwenden kann, erscheint es mir notwendig, sie mit einem neuen Ausdruck zu belegen. Ein solcher, der mir sehr adäquat erscheint, ist *Mikrotypus*. Dieser besagt, dass wir es mit den *kleinsten unterscheidbaren* Varianten zu tun haben, und nicht mehr. Er hebt ausserdem durch den »Typus«-Begriff hervor, dass diese keine kontinuierliche, untrennbare Reihe bilden, sondern als *habituell* gut umschriebene Varietäten je für sich gekennzeichnet werden können. *Eine grössere Population, Taraxacum officinale, kann also bei genauem Durchforschen durch Spezialisten restlos in ihre Mikrotypen aufgelöst werden.*

Aber weshalb, kann man nun einwenden, ist es bei *Taraxacum* möglich, Mikrotypen zu unterscheiden? Ist es nicht paradoxal, dass man bei einer Spezies, die eine ausserordentliche Variabilität aufweist, eine ungeheure Anzahl von Varianten unterscheiden kann, während man bei der Mehrzahl der Arten des Pflanzenreiches nur eine fast kontinuierliche Variation beobachtet, und in diesem Falle die wenigen, markierteren Varianten gar nicht zu bestimmten Typen zusammenfassen kann?

Die Erklärung dieser früher ganz unverständlichen Tatsache ist indessen gewiss auch in der besonderen Vermehrungsweise bei *Taraxacum* zu suchen. Sie ist nur die Folge der agamospermen Samenbildung.

Bei einer agamospermen Pflanzenart muss die Selektion eine sehr viel effektivere Wirkung haben als bei einem sexuellen Fremdbefruchter. Bei letzterem ist die Kombination frei; die Biotypen müssen ganz kaleidoskopisch entstehen und verschwinden. Die schwächeren Biotypen werden wohl ausgemerzt, aber sie werden durch die Gameten anderer Pflanzen der Population wieder neugebildet. Ihr Gametentypus »parasitiert« an den vitalen Pflanzen. Die Variabilität wird sehr zähe in ihrem ganzen Umfang festgehalten. Nur sinkt natürlich die Frequenz der avitalen Typen. *Vollkommen* ausgemerzt werden sie, wie eine theoretische Überlegung zeigt, niemals.

Eine ganz andere Wirkung hat dagegen die Selektion an einer Spezies, die agamosperme Fortpflanzung erhalten hat. Die Agamospermie ist bei den Kompositen, wie schon MENDEL (1869) für *Hieracium* gezeigt hat und wie OSTENFELD (1910) später bestätigen konnte, dominant. Wird also ein teilweise agamospermer Biotypus mit einem sexuellen gekreuzt, so wird die ganze Nachkommenschaft agamo-

sperm. Hieraus folgt, dass die agamospermen Biotypen in der Population (Spezies) mehr und mehr zunehmen müssen, bis endlich ihr ganzer Biotypeninhalt von der Agamospermie »fixiert« worden ist. Alle Biotypen sind dann »konstant« und geben nur ganz identische Nachkommen; sie sind zu Klonen geworden.

Nun setzt die Selektion mit einer gewaltigen Wirkung ein. Denn alles was nicht vital ist wird *definitiv* ausgemerzt. Alle schwachen Biotypen verschwinden. Ihre Neubildung ist ausgeschlossen, denn es findet kein sexueller Prozess mehr statt. Nach und nach gehen neue Biotypen zu Grunde, die nicht ganz konkurrenzkräftig sind. Die Spezies, die ursprünglich als sexuell vielleicht Millionen von verschiedenen Biotypen gehabt hat — diese Anzahl wird schon durch die Kombination von 20 Genen gebildet — verarmt allmählich an Biotypen. Diese Verarmung schreitet schnell vorwärts, reduziert die Million auf Hunderttausend, Hunderttausend auf Tausend und Tausend auf einige Hundert.

Und was geschieht dann? Ja, die *verbleibenden* Biotypen beginnen *morphologisch sichtbar* zu werden. *Die Spezies ist polymorph geworden.* Aber weshalb? Weil ihre ursprüngliche, sehr *grosse* Variabilität *ausserordentlich* stark *herabgesetzt* worden ist. Dies ist der wirkliche Gang der Erscheinung der Polymorphie! Die Polymorphie beruht nicht auf einer Vermehrung der Varianten, sondern auf einer ganz katastrophalen Verminderung ihrer Anzahl.

Was ist es also, das in einer polymorphen Art übrig bleibt? Die Biotypen. Ganz wie früher nur die Biotypen. Aber nur gewisse und sehr wenige Biotypen. Die vitalen Biotypen. Nun sehen wir klar, wie unsinnig es ist, diese dann Spezies zu nennen.

Wahrscheinlich bezeichnen die 450 unterschiedenen skandinavischen *Taraxaca* den ganzen oder sehr nahe den ganzen Biotypeninhalt der alten Spezies *T. officinale*. Nur weil es aus den oben angeführten Gründen nicht als zulässig angesehen werden kann, den nur in streng konstitutioneller Bedeutung verwendbaren Ausdruck Biotypus als morphologische Bezeichnung zu benutzen, wählen wir für diese wahrscheinlichen Biotypen den Ausdruck Mikrotypen. Mit dem sorgfältigen Feststellen der *Mikrotypen* einer *vollständig agamospermen Spezies* haben wir auch, jedenfalls sehr nahe, ihren *Biotypeninhalt* ermittelt.

Wir haben also nunmehr einen ganz klaren Einblick in die Ursachen der Polymorphie gewisser Arten und Gattungen. Wir wissen, dass ihre Komponenten nichts anders als Restbiotypen sind. In diesem Punkt ist also eine weitere Erörterung, die ihre »Rangordnung« feststellen sollte, ganz überflüssig. Sie sind die *kleinsten* Bausteine der

Spezies. Sie haben keinen Rang. Ebensowenig wie die Ziegelsteine eines Hauses vor dem Bauen einen Rang von differenzierten Teilen des Gebäudes haben.

Es verbleibt nun die Frage, ob die Population *Taraxacum officinale* als eine einzige Spezies, *T. officinale* WEB., aufgefasst oder ob diese in weitere Formenkreise, die pflanzengeographisch, ökologisch und zytologisch mehr mit den Artpopulationen allogamer Spezies übereinstimmen, zergliedert werden soll. Ich möchte hier nicht näher auf diese Frage eingehen — ich werde sie im Zusammenhang mit anderen »kritischen« Genera in einer grösseren Arbeit eingehender behandeln — sondern nur andeuten, dass ich im allgemeinen die Subgenera DAHLSTEDTS als Spezies betrachte (also *T. vulgare*, *palustre*, *ceratophorum* etc.). Eine zytologisch-ökologische Begründung hierfür hat GUSTAFSSON (1935) veröffentlicht. Eine genisch-experimentelle ist wegen der Agamospermie ganz ausgeschlossen.

Eine Aufteilung einer Spezies in ihre Mikrotypen ist natürlich nur bei agamospermen oder überhaupt bei apomiktischen Arten durchführbar. Schon die amphimiktischen Autogamen sind gewöhnlich keiner so stark selektiven Elimination von Biotypen ausgesetzt wie die Agamospermen, weil Kreuzungen zufällig vorkommen, durch die dann neue Biotypenschwärme geschaffen werden. Dass jedoch, wenn Vizinismus selten stattfindet, Variabilitätsverhältnisse auftreten, die sehr auffallend an die von *Taraxacum* erinnern, das zeigen die seit JORDAN bekannten mikrotypenähnlichen Erscheinungen bei *Erophila verna* (L.) F. CHEV. Das Zerfallen einer *vollständig* autogamen Spezies in Mikrotypen ist eine ebenso absolute Notwendigkeit wie das einer *vollständig* agamospermen. Die genische und selektive Konsequenz führt in beiden Fällen auf verschiedenen befruchtungsbiologischen Wegen zum selben Ziel (HERIBERT NILSSON, 1930).

Die selektive Verarmung des Biotypeninhalts von *Taraxacum*, wie sie oben dargestellt worden ist, ist natürlich nur unter der Voraussetzung gültig, dass keine Neubildung von Mikrotypen stattfindet.

In bezug auf diese Frage scheinen die Taraxakologen durch ihre Feldstudien zu der Auffassung gekommen zu sein, dass die Mikrotypenzahl begrenzt ist und dass neue Typen nicht auftreten. Vor allem hat MARKLUND (1940) in einer umfassenden Arbeit diese Ansicht bestimmt vertreten. »Unter den Arten«, hebt er hervor, »die in Nyland sicher oder möglicherweise in natürlichen Pflanzenvereinen heimisch sind, gibt es, wie aus dieser Darstellung hervorgegangen ist, gar keine im Untersuchungsgebiet oder überhaupt in Finnland endemischen. Nichts

deutet somit darauf hin, dass die in natürlicher Vegetation gedeihende *Taraxacum*-Flora des untersuchten Gebietes während der postglazialen Zeit den aller geringsten Zuschuss durch Neuentstehung erhalten hätte» (l. c., p. 36). Nur an ausgeprägten Kulturstandorten findet er eine nicht unbeträchtliche Anzahl von Mikrotypen, die nirgendwo ausserhalb des Gebietes gefunden worden sind. Schon ihr Auftreten betrachtet er als sehr bezeichnend dafür, dass sie eingeschleppt worden sind.

Das Resultat der Feldstudien von MARKLUND scheint also die Auffassung zu bestätigen, dass die selektive Verarmung an Biotypen nicht durch eine Neubildung von solchen kompensiert wird. Die Depauperierung der Population an Varianten ist endgültig, ganz wie man dies auf Grund von genischen Überlegungen zu erwarten hat.

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Bei Abschluss meines Manuskripts erhielt ich die sehr schöne experimentelle Arbeit von SØRENSEN und GUDJÓNSSON (1946) über *Taraxacum*. Sie führen ganz unwidersprechlich den Beweis durch, dass auch jetzt neue Mikrotypen entstehen. Vor allem weisen sie das Auftreten von Chromosomenvarianten, Aberranten, nach, wie sie diese mit einem von WINGE eingeführten Ausdruck nennen.

Diese Aberranten entstehen durch das Wegfallen oder Hinzukommen eines, in gewissen Fällen mehrerer Chromosomen. Da die diploide Zahl 24 ist, haben die neuen Formen  $24 - 1$ ,  $24 - 2$ ,  $24 - 3$ ,  $24 - 4$ ,  $24 - 5$ ,  $24 + 1$ ,  $24 + 2$ . Ausserdem wurden Polyploide gefunden, die sowohl Verdoppelung der Grundzahl (also  $2n = 48$ ) wie aneuploide Verdoppelung  $2n = 2(24 - 1) = 46$  usw. zeigten. Hierdurch erhält man eine sehr ergiebige Quelle für die Produktion neuer Formen.

Die erwähnten Forscher haben auch, besonders für die Zahl  $2n = 23$ , bewiesen, dass wirklich alle erwarteten Aberranten auftreten. Da die Grundzahl  $n = 8$  ist — was GUDJÓNSSON durch das Auftreten von 3 ganz ähnlichen Gruppen von Chromosomen im Karyotypus zytologisch festgestellt hat — so sind 8 verschiedene Aberranten zu erwarten. Sie sind auch alle gefunden und beschrieben worden.

Was ist nun die weitere Konsequenz dieser chromosomalen Verhältnisse? Natürlich die, dass *sämtliche* diese vielen Aberranten bei *allen* Mikrotypen vorkommen können. Mit anderen Worten: Jeder Mikrotypus kann sich mit jeder Aberrante kombinieren. Von Mikrotypus *pynolobum* hat man z. B. 8 Aberranten. Aber auch bei den übrigen konstatierten, sagen wir 449, Mikrotypen sind von jedem 8 neue

Formen zu erwarten. Das macht schon 3:592. Aber dazu kommen neue Aberranten von den neuen aneuploiden Zahlen (24 — 2, usw.) und neue Polyploide und aneuploide Polyploide. Die mögliche Anzahl neuer Aberranten steigt damit fast in das Unermessliche.

Diese Resultate sind experimentell begründet. Dass also eine *überaus reiche Neubildung* von Biotypen innerhalb der Mikrotypen stattfindet ist *sicher*.

Wie lassen sich nun diese Resultate mit den ganz entgegengesetzten der Taxonomen vereinigen? Und weiter fragt man: Weshalb haben die Taraxakologen, die oft Kulturversuche ausgeführt haben, nichts von dieser Erscheinung beobachtet?

Eine gewiss richtige Erklärung für die letztgenannte Diskrepanz geben SØRENSEN und GUDJÓNSSON in ihrer Arbeit (p. 7): teils hat man nur winzige Nachkommenschaften aufgezogen, teils sind beim Auspflanzen gewiss die kräftigeren Pflanzen ganz unbewusst ausgelesen worden. Meine Methodik kränkelte gewiss an diesen beiden Fehlerquellen. Aber meine Versuche bezweckten nicht *neue* Varianten aufzusuchen, sondern ich wollte nur die *vorhandene* Variabilität feststellen. Als Untersuchungen über eine Formenneubildung haben sie deshalb keinen Wert.

Von den SØRENSENSchen Aberranten habe ich nur eine einzige gefunden. In einer Nachkommenschaft von *polyodon* trat 1934 eine abweichende, diffus gelbblättrige Pflanze auf. Wie ich jetzt sehe, stimmt sie sehr gut mit der Aberrante *olivacea* überein. Diese trat auch in den Versuchen von SØRENSEN gerade bei *polyodon* »fairly frequently« auf.

Noch eine abweichende Form, die mit keiner seiner erwähnten Aberranten übereinstimmt, trat in einer Nachkommenschaft von *fulvum* auf. Die Pflanze wich von ihrem Mikrotypus durch ihren sehr üppigen Wuchs ab. Das Format war in bezug auf alle Organe das doppelte. Fig. 3 zeigt dies sehr gut. Rechts sieht man eine Parzelle des *fulvum*-Mikrotypus, links eine ihres Abweichers. Der Unterschied ist auffallend.

Durch das Aussehen des Abweichers war ich sogleich fest davon überzeugt, dass hier eine polyploide Form vorlag. Leider wurde keine Chromosomenbestimmung ausgeführt. Meine Versuche wurden 1939 abgebrochen, weil ich das mir gesetzte Ziel damals als erreicht betrachtete. Als ich 1945 die Versuche mit *fulvum* und ihrer abweichenden Form wiederaufnehmen wollte, zeigte es sich, dass die Samen nicht mehr keimfähig waren.

Die Resultate von SØRENSEN zeigen nun, dass die Polyploide gar

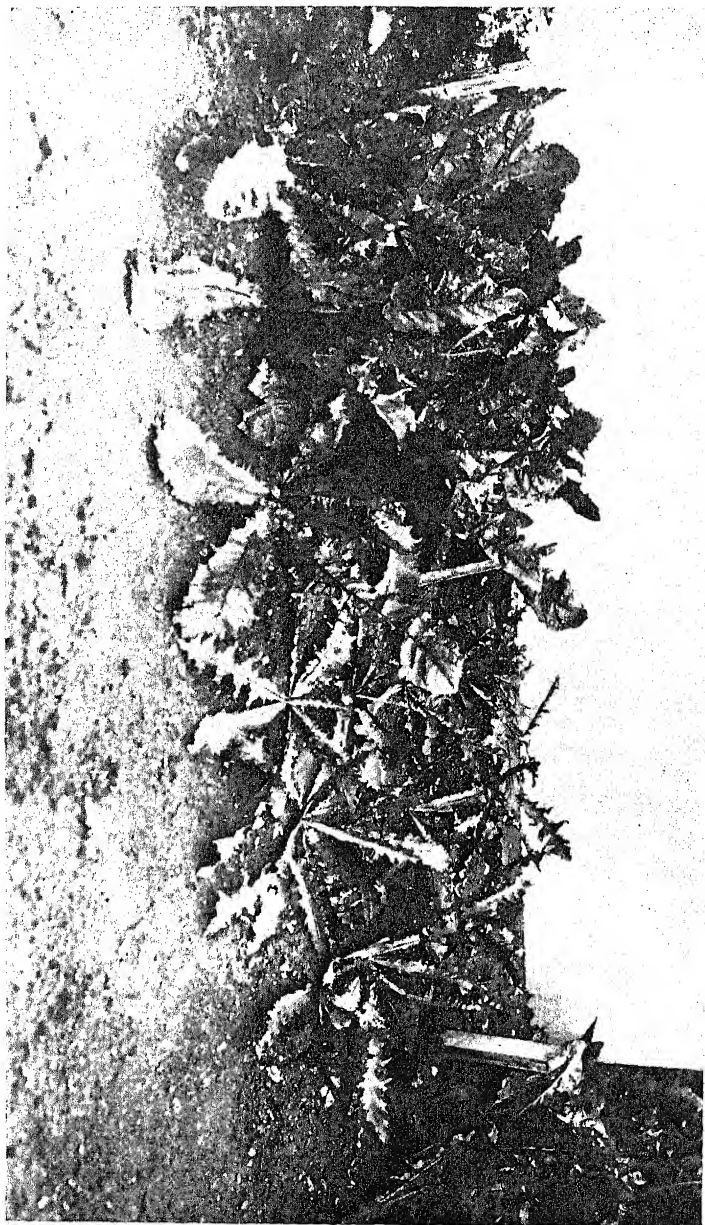


Fig. 3. Der Mikrotypus fulvum (rechts) und sein giganteum-Abweicher (links).

nicht das Aussehen meines *giganteum*-Typus haben. Sie sind kleiner als ihre Muttermikrotypen, sie haben einen gedrungenen Wuchs. Die Blütenköpfchen sind indessen auffallend gross. Die Pflanze ist deshalb sehr disproportioniert. Von einem Gesamtriesen wie bei meinem *giganteum*-Abweicher ist dort keine Rede. Dieser muss daher eine Neuvariante ganz anderer Natur darstellen als der einer Tetraploide (Hexaploide). Leider ist nun nicht einmal das Ermitteln ihrer Chromosomenzahl mehr möglich.

Dass der *giganteum*-Abweicher aus *fulvum* hervorgegangen ist, darüber besteht kein Zweifel. Er hat nicht nur den Habitus dieses Mikrotypus, nur im grösseren Format, beibehalten. Er hat auch die sehr charakteristische Samenfarbe dieses Typus, die nicht ziegelrot, wie bei der Mehrzahl der übrigen *Erythrosperma*, sondern hell gelbrot (terrakotta) ist. Ferner ist keine ähnliche Form unter den schwedischen *Taraxaca* bekannt.

Durch die Untersuchung von SØRENSEN und GUDJÓNSSON ist also festgestellt, dass bei *Taraxacum* sehr häufig chromosomale Varianten auftreten. Für die beiden am ausführlichsten untersuchten Mikrotypen, *laciniosifrons* und *polyodon*, geben sie den Prozentsatz 2,1 resp. 1,3 an. Aber einzelne Mikrotypen zeigen eine noch stärker erhöhte Aberrantenproduktion. Für *protractifrons* finden sie 8 %, für *expallidiforme* 4,2 %. Bei anderen Mikrotypen war der Prozentsatz dagegen viel niedriger; bei *bracteatum* 0,2 % und bei *copidophyllum* 0,1 %.

Diese überaus reiche Produktion von Aberranten, also auch von neuen Mikrotypen, scheint meine oben angeführte Ansicht über den Effekt der selektiven Verarmung bei *Taraxacum* umzustürzen. Denn man muss wohl annehmen, dass die Selektion durch diese reiche Mikrotypenneubildung kompensiert werden sollte.

Das ist aber keineswegs sicher. Wie ich sogleich zeigen werde, ist es ganz unwahrscheinlich.

Studiert man die Angaben von SØRENSEN über die Vitalität der Aberranten, so findet man, dass sie schwächer sind als ihre Muttermikrotypen. So ist z. B. bei *elegans* »the fertility noticeably reduced, the heads always containing a relatively large number of empty achenes«. Über *truncata* heisst es: »This aberrant shows some tendency to collapse during the flowering, giving rise to 'gaps' in the rows»; und weiter: »The fertility is somewhat reduced, a great many empty or poorly developed achenes being always present«. Über *hamosa* wird berichtet, dass »after the flowering and ripening of the fruits in the second or third year a larger or smaller number of the plants will die

without any previous symptoms of weakness». Die Aberrante *olivacea* hat, wie oben erwähnt, diffus gelbgefleckte Blätter. Sie hat zarteren Wuchs und kleinere Köpfchen als ihr Muttermikrotypus, was wohl mit einer herabgesetzten Assimilation der gelblichen Blätter einhergeht. Die *crassifolia* bildet keineswegs eine kräftige Pflanze. Sie ist kleinwüchsig mit sehr kleinen Köpfchen, die ein auffallend grosses Involucrum haben. Ihre Blätter sind klein, aber sehr dick. *Tenuis* bildet eine kleine, fast sterile Pflanze und *pygmaea* ist ein Zwerg mit einem Wurzelsystem wie ein Hexenbesen.

Alle diese sieben Aberranten haben offenbare konstitutionelle Schwächen, die ihre Vitalität stark herabsetzen und ihre Konkurrenzkraft auf ein Minimum reduzieren.

Nur eine der primären Aberranten ( $2n - 1$ ) ist kräftig und scheint in bezug auf Bau und Fertilität ihrem Muttermikrotypus gleichwertig zu sein, nämlich *plumosa*. Aber auch diese zeigt Eigenschaften, die auf eine disharmonische Konstitution hinweisen. Die Blätter sind z. B. konvex hinübergebogen, etwas gekräuselt, was eine nicht ungewöhnliche Erscheinung bei Mutanten mit herabgesetzter Vitalität (z. B. bei *Oenothera*) ist.

Dass sämtliche diese Aberranten eine geringe Konkurrenzkraft haben, geht auch aus der Tatsache hervor, dass sie von SØRENSEN nur einige wenige Male in der Natur gefunden worden sind. Sie sind dort also sehr selten. *Truncata* hat er dort nur viermal angetroffen, *elegans* zweimal, *plumosa* einmal. Das ist alles.

Dass die Aberranten von SØRENSEN eine so geringe Konkurrenzkraft besitzen, dass ihr Selektionswert auf Null sinkt, wird dadurch bestätigt, dass sie den Taraxakologen aus der Natur unbekannt sind. Skandinavische *Taraxacum*-Forscher, die sich lebenslang mit der Unterscheidung der kleinsten Varianten in der Natur beschäftigt haben und die ein ausserordentlich geübtes Auge für neuauftauchende Formen besitzen, kennen die grosse und auffällige Variabilität in den Kulturen von SØRENSEN nicht. Hierfür gibt es nur eine einzige Erklärung. Sie sind dort nicht zu finden. Sie werden nach ihrer Entstehung sehr schnell wieder ausgemerzt.

Natürlich können die Aberranten in der Natur ebenso entstehen wie im Versuchsgarten von SØRENSEN. Es erscheint wohl ausgeschlossen, dass das Versetzen von *Taraxaca* in einen Garten eine Mutabilität auslösen würde. *Taraxacum* ist ja schon stark kulturgebunden, jedenfalls die *Vulgaris*-Formen. Zufällig kann man sie in der Natur antreffen, aber sehr selten, wie ich oben berichtet habe. SØRENSEN

erwähnt auch (l. c., p. 15), dass LINDBERG FIL. ein einzigesmal seine Aberrante *plumosa* gefunden und sie auch kultiviert hat. Das zeigt ja, dass das Unterscheiden der Aberranten den Taraxakologen keine Schwierigkeit bereitet. Aber es kommt nur ganz zufällig vor, dass sie eine solche Pflanze zu sehen bekommen, weil die Aberranten sich nur ganz ohne Konkurrenz durchsetzen können. An zufällig vegetationsfreien Flecken können ihre Samen keimen und sich zu Pflanzen entwickeln. Sobald ein konkurrierender Bestand aufwächst, verschwinden sie.

Aus den angeführten Tatsachen ergibt sich, dass die grosse und auffallende Produktion von neuen Mikrotypen, die durch die Versuche von SØRENSEN und GUDJÓNSSON nachgewiesen worden ist, meine Schlüsse in bezug auf die natürliche Variabilität, den Biotypeninhalt und die selektive Verarmung bei *Taraxacum* ganz unberührt lassen. Falls ihre Ergebnisse meine Schlussfolgerungen beeinflussen, so ist es in positiver Richtung.

Man muss nämlich sagen, dass die neuen Tatsachen die Auffassung des selektiven Ausmerzens grosser Mengen von Biotypen beweisen. Sie beweisen nämlich, dass auch ganze Schwärme von Mikrotypen, von deren Existenz wir bis jetzt nichts wussten, *auftreten* und *verschwinden* wie ein Rauch im Sturm. In ähnlicher Weise verschwand auch die Mehrzahl der rekombinierten Biotypen, nämlich alle nicht vollitalen, sobald die Variabilität durch die Agamospermie fixiert worden war. Sie waren durch das rekombinierende Spiel nicht mehr zu retten. Und die jetzt noch entstehenden neuen Mikrotypen sind schon vom Anfang an dem selektiven Ausmerzen preisgegeben. Sie sind wohl übrigens auch nicht neu, sondern genische Neukombinationen, die durch die Konstitution ihrer Spezies ursächlich und gesetzmässig bedingt sind. Sie erscheinen deshalb immer wieder von neuem. Und sie sind gewiss ebenso alt wie die Spezies.

Die oben angeführten Tatsachen und Schlussfolgerungen dürften wohl zeigen, dass zur Lösung des Problems der polymorphen Spezies in keinem Falle besondere Variabilitätserscheinungen angenommen werden brauchen. Die Ursache der grossen Anzahl unterscheidbarer Varianten beruht nämlich nicht, wie früher angenommen wurde, auf einer reichen Neubildung vitaler Varianten, sondern im Gegenteil auf einer ausserordentlich scharf selektiven Elimination von Biotypen der Speziespopulation. Die Restbiotypen treten dann, weil sie von Millionen bis auf Hunderte reduziert worden sind, für das Auge — jedenfalls der Spezialisten unter den Taxonomen — zutage. Was sie aber als Neu-

heiten beobachten, und was viele Artbildungstheoretiker mit Enthusiasmus als Zeichen einer Artbildung im Fluss auffassen, ist uralte.

### SUMMARY.

Title of paper: *Total inventorization of micro-types on a minimum area of Taraxacum officinale.*

The taxonomists have distinguished about 450 so-called »micro-species» of *Taraxacum officinale* WEB. in Scandinavia. A natural question is then whether the variation is exhausted with this, or whether these micro-species are collective or bridging forms exist between them.

For the purpose of solving this question the author collected from a limited area, only 50 square metres, seeds from *all* the *Taraxacum* plants growing there. These consisted of 177. On cultivation in plots followed by repeated seed-gathering and cultivation for five years it was found that 20 distinct types occurred on this minimum area, 19 of which represented previously described »micro-species» and only one was new. There was *no* variation round or between these types, the whole variation being exhausted with these distinct types. Of e. g. the *pycnolobum* »micro-species», which was represented by 51 initial plants, all plots in the progeny were perfectly alike. At the repeated tests undertaken not the slightest variation could be discovered between the 51 plots or between the plants within a plot.

The morphological conformity was accompanied by a physiological one. Within one »micro-species» there was no variation as regards rhythm of development, flowering time or nyctitropism, but an extremely marked such between different »micro-species».

It is therefore probable that »micro-species» are to be regarded as biotypes, distinct biotypes, between which there are no bridging forms. The number of biotypes in the Scandinavian population of *Taraxacum officinale* WEB. would thus not seem to be more than about 500. Just because the biotype number is so small specialized taxonomists are able to distinguish them.

As »micro-species» are biotypes, it is impossible to regard them as species. They are the least conceivable variants in a species. Naturally they cannot then be species themselves. They are constant, but this is on account of their agamospermous reproduction. They are clones, and if we were to call every such in the vegetable kingdom a species we should have to calculate these in at least billions, i. e. in astronomical figures.

Highly probably the distinguished morphological types are really biotypes. This genetic term, however, is hardly serviceable in taxonomy. For it means absolute constitutional identity. As we cannot declare with full certainty on the basis of ocular judgment that absolutely identical individuals are always brought together under our name of a type, it is necessary to create a new term for the least *distinguishable* units. I suggest the term *micro-type* for such. Judging from my results, however, it seems an indubitable fact that the micro-types in *Taraxacum* are biotypes.

*T. officinale* is a polymorphic species, and such a species has always been considered to be extremely variable. But this is not the case. Instead, the number of micro-types is strikingly small. Even at only 20 character differences over 1.000.000 biotypes are formed in a cross-fertilizer. About 500 in *Taraxacum* is an extremely small number.

That the number of micro-types has fallen so much in *Taraxacum* is an inevitable consequence of its agamospermous propagation. All micro-types that are not fully vital are eliminated through selection, while in a cross-fertilizer they persist in a certain percentage on account of panmixia. In an agamospermous species swarms of biotypes will be *entirely* obliterated, and hence the number must successively fall until only the fully vital are left. These then appear as distinguishable, i. e. as micro-types, as a consequence of the selective breakdown of the former continuous chain of variation.

However paradoxically it may seem, a polymorphic species will therefore be critical on account of its low variability. Polymorphism is by no means caused by the fact that a lively formation of species goes on, as is usually held, but by a mass death.

The rich production of chromosomally different forms out of *Taraxacum* micro-types found by SØRENSEN and GUDJÓNSSON does not affect the results and conclusions presented here, as these forms are not vital and are not to be found in nature except by pure chance.

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# THE GENETIC EFFECTS OF BREEDING IN SMALL POPULATIONS

## A DEMONSTRATION FOR USE IN GENETIC TEACHING

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WHEN teaching Elementary Science it is always important to arrange the demonstrations and the experiments in such a way that the essentials are brought out as obviously as possible. But the obstacles may be numerous. For instance in Genetics, when the question is to demonstrate the homozygotizing effect of inbreeding in allogamous species, the difficulties are very great if it is desired to use living material: the necessarily great number of generations are impossible to come up to during the limited space of time, absolute fertility equality of the different animals is practically never fulfilled, and really free intercrossings are very difficult to ensure. And even if all these practically unsurmountable difficulties could be overcome, the effect of dominance (and of epistasis in the case of two or more pairs of genes) would still make it impossible to determine exactly the number of generations after which all heterozygosity has been eliminated.

As it must nevertheless be looked upon as very important in Genetics class work to demonstrate the principal effects of inbreeding, other ways must be sought. To give WRIGHT's mathematical deductions (1921 a, 1931) would, however, usually be far above the horizon of the auditors. And if the demonstration is confined to the exposition of his well-known curves (1921 b, 1931), the most important part, viz. the effect of random causes, would be difficult to make clear. In my Genetics class, therefore, I have for a number of years made use of a kind of roulette-game. Of course, any other machinery than a roulette may likewise be used if only all the necessary probabilities can be covered. A table of random numbers may also be useful. This roulette system has been very successful, and as it may be of some value for other teachers in Genetics it will be demonstrated here.

The demonstrations have been confined to the case of 1 pair of genes,  $A$  and  $a$ . Instead of free intercrossing it is thought that every generation is propagated by a limited number of monogamous parental pairs. The principal results will be the same in both cases, and it is simpler to work with pairs of monogamous parents than with free intercrossing. Suppose, thus, that in each generation there are  $n$  pairs of parents and that of these, in a certain generation, there are

$$\begin{array}{ll} n_1 \text{ pairs} & AA \times AA \\ n_2 & \gg AA \times Aa \\ n_3 & \gg AA \times aa \\ n_4 & \gg Aa \times Aa \\ n_5 & \gg Aa \times aa \\ n_6 & \gg aa \times aa \end{array}$$

By working out the kind of progeny from each type of parental combination it will immediately be found that the proportions of the three genotypes  $AA$ ,  $Aa$ , and  $aa$  in the next generation are

$$\frac{4n_1 + 2n_2 + n_4}{4n}, \frac{2n_2 + 4n_3 + 2n_4 + 2n_5}{4n}, \frac{n_4 + 2n_5 + 4n_6}{4n} \quad (1)$$

These three ratios thus also give the probabilities that a parent within the next generation will belong to one or other of the three possible genotypes. And these probabilities are — at least for the smallest values of  $n$  — easy to realize with the aid of a roulette.

In the Genetics class the students usually have to work out the cases of  $n=1$ , 2, and 5. They are therefore given sheets containing working tables. Tables 1 and 2 show these working tables for  $n=1$

TABLE 1. *Working table for  $n=1$  (brother—sister matings).*

Parental generation						Progeny generation					
$n_1$	$n_2$	$n_3$	$n_4$	$n_5$	$n_6$	Probability in fourths of			Roulette		
						$AA$	$Aa$	$aa$	$AA$	$Aa$	$aa$
1	0	0	0	0	0	4	0	0	all	—	—
0	1	0	0	0	0	2	2	0	1—18	19—36	—
0	0	1	0	0	0	0	4	0	—	all	—
0	0	0	1	0	0	1	2	1	28—36	10—27	1—9
0	0	0	0	1	0	0	2	2	—	1—18	19—36
0	0	0	0	0	1	0	0	4	—	—	all

TABLE 2. Working table for  $n=2$ .

Parental generation						Progeny generation					
$n_1$	$n_2$	$n_3$	$n_4$	$n_5$	$n_6$	Probability in eighths of			Roulette		
						AA	Aa	aa	AA	Aa	aa
2	0	0	0	0	0	8	0	0	all	—	—
1	1	0	0	0	0	6	2	0	1-27	28-36	—
1	0	1	0	0	0	4	4	0	19-36	1-18	—
1	0	0	1	0	0	5	2	1	1-20	21-28	29-32
1	0	0	0	1	0	4	2	2	19-36	10-18	1-9
1	0	0	0	0	1	4	0	4	1-18	—	19-36
0	2	0	0	0	0	4	4	0	19-36	1-18	—
0	1	1	0	0	0	2	6	0	1-9	10-36	—
0	1	0	1	0	0	3	4	1	21-32	5-20	1-4
0	1	0	0	1	0	2	4	2	1-9	10-27	28-36
0	1	0	0	0	1	2	2	4	28-36	19-27	1-18
0	0	2	0	0	0	0	8	0	—	all	—
0	0	1	1	0	0	1	6	1	1-4	5-28	29-32
0	0	1	0	1	0	0	6	2	—	10-36	1-9
0	0	1	0	0	1	0	4	4	—	1-18	19-36
0	0	0	2	0	0	2	4	2	28-36	10-27	1-9
0	0	0	1	1	0	1	4	3	1-4	5-20	21-32
0	0	0	1	0	1	1	2	5	29-32	21-28	1-20
0	0	0	0	2	0	0	4	4	—	1-18	19-36
0	0	0	0	1	1	0	2	6	—	28-36	1-27
0	0	0	0	0	2	0	0	8	—	—	all

and 2, which are constructed so as to make the number of holes in the roulette, corresponding to the three genotypes in the offspring generation, to be in the proportions (1). As it is possible to combine the integers  $n_1, n_2, n_3, n_4, n_5, n_6$ , the sum of which is  $n$ , in  $\frac{(n+5)}{n! 5!}$

different ways, the number of entries for  $n=1$  (brother—sister matings, Table 1) is 6, and for  $n=2$  (Table 2) the number of entries is 21. The students who work with 5 pairs of parents get a working table with  $\frac{10!}{5! 5!} = 252$  entries (not shown here).

When the experiment starts it is thought that the population consists of only heterozygous individuals, i. e. it is a  $F_1$  population. Therefore  $n_4=n$  and  $n_1=n_2=n_3=n_5=n_6=0$ . Suppose now we are dealing with the case of 2 pairs of parents per generation. From the working table (Table 2) the student finds that in the progeny ( $F_2$ )

after  $n_1=2$  the numbers of  $AA$ ,  $Aa$ , and  $aa$  individuals are (in the 8th) 2, 4, 2. He then makes two pairs of throws (i. e. in all 4 throws), the first corresponding to the first parental pair and the second to the second parental pair. Suppose that he gets the holes 9, 28, 17, 12 (in the given order). The table shows that for  $n_1=n_2=n_3=0$ ,  $n_4=2$ ,  $n_5=n_6=0$ , number 9 denotes  $aa$  and number 28  $AA$ . The first parental pair taken from the  $F_2$  is thus an  $AA \times aa$  cross. Likewise 17 denotes  $Aa$  and 12 also  $Aa$ , and consequently the second parental pair in the  $F_2$  is an  $Aa \times Aa$  cross. The parents from the  $F_2$  correspond thus to  $n_1=n_2=0$ ,  $n_3=n_4=1$ ,  $n_5=n_6=0$ , and the table shows that in the progeny ( $F_3$ ) the numbers of  $AA$ ,  $Aa$ , and  $aa$  individuals are (in the 8th) 1, 6, 1. The student makes 2 new pairs of throws and, using the entry  $n_1=n_2=0$ ,  $n_3=n_4=1$ ,  $n_5=n_6=0$ , he finds what kind of parental cross he gets in the  $F_3$  and what proportions of  $AA$ ,  $Aa$ , and  $aa$  individuals in the  $F_4$ . He proceeds in this way until complete fixation, which occurs when he gets either  $n_1=2$ ,  $n_2=n_3=n_4=n_5=n_6=0$  (fixation with  $AA$  individuals) or  $n_1=$   
 $=n_2=n_3=n_4=n_5=0$ ,  $n_6=2$  (fixation with  $aa$  individuals).

In the case of brother—sister matings only one pair of throws (i. e. in all 2 throws) is made per generation, and in the case of 5 parents per generation, 5 pairs of throws (i. e. in all 10 throws) are made per generation. It is to be remarked that if a certain number is not included among the holes of the roulette, as for instance zero, and if this number comes out after a throw, this throw is passed as if it had not been made.

The students have to make careful records of the results of each generation. They have also to make a number of parallel series of similar kind, just in order to study the great randomly determined variability in different series. Usually they have to make 100 series. For each generation they also make out the totals of  $AA$ ,  $Aa$ , and  $aa$  individuals (in the 4nth) and divide this total by the number of series  $\times 4n$ , whereby they get the average proportions of the three kinds of genotypes. In the Genetics class of 1946 the students made 100 parallels of each of the cases  $n=1$ ,  $n=2$  and  $n=5$ , and the final records of these experiments are given in Tables 3, 4 and 5. These tables show the average heterozygosity and also — and this is perhaps the most important — the total number of series which up to each generation have been fixed as  $AA$  or as  $aa$ . The result thus clearly shows: (1) The *average* heterozygosity decreases, at first at a rapid rate, later more slowly, and vanishes completely after a rather

TABLE 3. *Record for 100 series with  $n=1$ .*

Generation	$F_2$	$F_3$	$F_4$	$F_5$	$F_6$	$F_7$	$F_8$	$F_9$	$F_{10}$	$F_{11}$	$F_{12}$	$F_{13}$
Proportion of $Aa$												
Observed	0,5000	0,5100	0,3600	0,2900	0,2200	0,1650	0,1550	0,0950	0,0800	0,0600	0,0600	0,0450
Expected	0,5000	0,3750	0,3125	0,2500	0,2035	0,1641	0,1328	0,1074	0,0869	0,0705	0,0569	0,0460
Total number of series which have been fixed												
as $AA$	—	5	14	23	31	38	44	45	47	49	49	50
as $aa$	—	7	15	21	24	30	31	34	36	39	41	41
Generation	$F_{14}$	$F_{15}$	$F_{16}$	$F_{17}$	$F_{18}$	$F_{19}$	$F_{20}$	$F_{21}$	$F_{22}$	$F_{23}$	$F_{24}$	$F_{25}$
Proportion of $Aa$												
Observed	0,0300	0,0150	0,0100	0,0100	0,0100	0,0050	0,0050	0,0050	0,0050	0,0100	0,0050	0,0050
Expected	0,0372	0,0301	0,0244	0,0197	0,0159	0,0129	0,0104	0,0084	0,0068	0,0055	0,0045	0,0036
Total number of series which have been fixed												
as $AA$	52	53	53	53	53	54	54	54	54	54	54	54
as $aa$	42	44	45	45	45	45	45	45	45	45	45	45
Generation	$F_{26}$	$F_{27}$										
Proportion of $Aa$												
Observed	0,0050	0,0000										
Expected	0,0029	0,0024										
Total number of series which have been fixed												
as $AA$	54	55										
as $aa$	45	45										

great but limited (as long as the number of parallel series is limited) number of generations. (2) This rate of decrease in heterozygosity is very different in different series and the variation is due to random causes. In the case of 2 parents per generation, for instance (Table 4), one series was completely homozygous already in the  $F_3$ , whereas one series did not get rid of its heterozygosity until the  $F_{62}$ . It is also due to random causes if the fixation should be in respect to  $A$  or to  $a$ . (3) *On an average* it takes more generations in the case of  $n=5$  to get completely rid of heterozygosity than in the case of  $n=2$  or



TABLE 5. Record for 100 series with  $n=5$ .

G e n e r a t i o n	F <sub>2</sub>	F <sub>3</sub>	F <sub>4</sub>	F <sub>5</sub>	F <sub>6</sub>	F <sub>7</sub>	F <sub>8</sub>	F <sub>9</sub>	F <sub>10</sub>	F <sub>11</sub>	F <sub>12</sub>	F <sub>13</sub>	F <sub>14</sub>	F <sub>15</sub>	F <sub>16</sub>	F <sub>17</sub>	F <sub>19</sub>
Proportion of Aa Observed .....	0.500	0.500	0.495	0.432	0.384	0.356	0.338	0.305	0.283	0.263	0.237	0.226	0.211	0.202	0.178	0.157	0.146
Total number of series which have been fixed as AA	—	—	—	—	2	3	5	7	10	11	14	15	17	19	22	27	28
as aa	—	—	—	1	2	4	5	9	15	16	19	23	25	26	29	31	32
G e n e r a t i o n	F <sub>19</sub>	F <sub>20</sub>	F <sub>21</sub>	F <sub>22</sub>	F <sub>23</sub>	F <sub>24</sub>	F <sub>25</sub>	F <sub>26</sub>	F <sub>27</sub>	F <sub>28</sub>	F <sub>29</sub>	F <sub>30</sub>	F <sub>31</sub>	F <sub>32</sub>	F <sub>33</sub>	F <sub>34</sub>	F <sub>35</sub>
Proportion of Aa Observed .....	0.144	0.134	0.127	0.115	0.109	0.116	0.109	0.088	0.079	0.075	0.063	0.066	0.067	0.058	0.065	0.043	0.056
Total number of series which have been fixed as AA	29	29	29	31	31	31	33	34	35	35	35	35	37	37	37	38	38
as aa	35	36	36	38	38	38	38	40	43	44	46	47	48	50	50	50	50
G e n e r a t i o n	F <sub>36</sub>	F <sub>37</sub>	F <sub>38</sub>	F <sub>39</sub>	F <sub>40</sub>	F <sub>41</sub>	F <sub>42</sub>	F <sub>43</sub>	F <sub>44</sub>	F <sub>45</sub>	F <sub>46</sub>	F <sub>47</sub>	F <sub>48</sub>	F <sub>49</sub>	F <sub>50</sub>	F <sub>51</sub>	F <sub>52</sub>
Proportion of Aa Observed .....	0.058	0.054	0.045	0.039	0.040	0.041	0.040	0.043	0.038	0.042	0.035	0.026	0.027	0.023	0.020	0.020	0.015
Total number of series which have been fixed as AA	38	38	38	39	39	39	39	39	40	40	40	40	40	42	43	44	44
as aa	50	50	50	50	50	50	50	50	50	50	50	51	51	51	51	51	51
G e n e r a t i o n	F <sub>53</sub>	F <sub>54</sub>	F <sub>55</sub>	F <sub>56</sub>	F <sub>57</sub>	F <sub>58</sub>	F <sub>59</sub>	F <sub>60</sub>	F <sub>61-64</sub>	F <sub>65</sub>	F <sub>66-67</sub>	F <sub>68</sub>	F <sub>69</sub>				
Proportion of Aa Observed .....	0.014	0.014	0.013	0.007	0.009	0.010	0.007	0.005	0.003	0.002	0.003	0.001	0.000				
Total number of series which have been fixed as AA	44	44	44	44	45	45	45	45	45	45	45	45	45				
as aa	51	52	52	52	52	52	53	53	54	54	54	54	55				

$n=1$ , and also more generations in the case of  $n=2$  than in the case of  $n=1$ . It is especially on point (2) that the teacher should lay particular stress, as it is this point which is practically impossible to demonstrate with living material or by average curves of heterozygosity. It is also these randomly caused variations which give rise to such an important phenomenon in Nature's household as the genetical drift.

Concerning point (1), it usually seems quite enough to emphasize the general trend in heterozygosity, whereas any comparisons between observed and expected frequencies seem, from a teacher's point of view, to be of less importance. Such expected frequencies are, however, given in Tables 3 and 4. In the case of  $n=1$  (brother-sister matings) they may be found, for instance, in WRIGHT's work (1921 b, p. 127) or in JENNINGS (1914). As will be seen there, the proportion of heterozygosity in the  $F_h$  is  $\frac{x_h}{2^h}$ , where  $x_h = \text{FIBONACCI's}$  number defined by the relations  $x_h = x_{h-1} + x_{h-2}$  and  $x_0 = x_1 = 1$ . A general formula from which it would be theoretically possible to compute these frequencies for any value of  $n$  is easy to find, but it is of very limited use, as the computational difficulties increase enormously already when  $n > 2$ . But for the sake of completeness the deduction of the formula will be shown here.

Suppose the frequencies of the zygotes  $AA$ ,  $Aa$  and  $aa$  to be  $x$ ,  $y$  and  $z$  ( $x + y + z = 1$ ). Then the 6 possible modes of pairings are obviously expected in the frequencies  $(x + y + z)^2 = x^2, 2xy, 2xz, y^2, 2yz, z^2$ . Consequently,  $n$  pairs must be distributed as the expansion of  $(x^2 + 2xy + 2xz + y^2 + 2yz + z^2)^n$ , i. e. the frequency of the combination  $n_1, n_2, n_3, n_4, n_5, n_6$  of the 6 possible pairings must be

$$\frac{n!}{n_1! n_2! n_3! n_4! n_5! n_6!} (x^2)^{n_1} (2xy)^{n_2} (2xz)^{n_3} (y^2)^{n_4} (2yz)^{n_5} (z^2)^{n_6} =$$

$$= \frac{n! 2^{n_2 + n_3 + n_5}}{n_1! n_2! n_3! n_4! n_5! n_6!} x^{2n_1 + n_2 + n_3} y^{n_2 + 2n_4 + n_5} z^{n_3 + n_5 + 2n_6}$$

If thus  $p_{n_1 \dots n_6}^{(h)}$  denotes this frequency in the generation  $h$ , the following formula of recurrence must hold good

$$p_{n_1 \dots n_6}^{(h)} = \frac{n! 2^{n_2 + n_3 + n_5}}{n_1! n_2! n_3! n_4! n_5! n_6!} \times$$

$$\times \sum_{s_1 \dots s_6}^{(h-1)} x^{2n_1 + n_2 + n_3} y^{n_2 + 2n_4 + n_5} z^{n_3 + n_5 + 2n_6} \dots \dots (2)$$

Here  $x, y, z$  have the values

$$x = \frac{4s_1 + 2s_2 + s_4}{4n}$$

$$y = \frac{2s_2 + 4s_3 + 2s_4 + 2s_5}{4n}$$

$$z = \frac{s_4 + 2s_5 + 4s_6}{4n}$$

and the summation is to be made for all the  $\frac{(n+5)!}{n! 5!}$  combinations of  $s$ 's for which  $s_1 + s_2 + s_3 + s_4 + s_5 + s_6 = n$ . The chief difficulty in the computations comes from the largeness of  $\frac{(n+5)!}{n! 5!}$ .

From the frequencies (2) the expected heterozygosity may be directly computed, and this has been done in Table 4 up to the  $F_{13}$ .

It is easy to construct working tables of similar kinds in order to demonstrate other effects of breeding in small populations. Thus, the Genetics class usually also makes roulette trials to demonstrate the combined effect of inbreeding and complete selection against a recessive.

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# THE INHERITANCE OF THE PLATINUM AND WHITE FACE CHARACTERS IN THE FOX

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## INTRODUCTION.

THE black fox, which is farm bred in the Scandinavian countries, is descended from animals imported from North America, where it has arisen from the wild red fox through mutation. The mutant gene in the homozygous condition changes the colour of the whole animal, in the same way as a mutation of the agouti gene in the wild type rabbit changes the colour of the whitish underline and the banded guard hairs to solid black. Two phenotypically similar but genetically different mutant types of black foxes are known, viz. the Alaskan and the standard fox. When Alaskan blacks are crossed with reds the  $F_1$  animals are intermediate in colour, showing the typical »cross fox» character, but the standard black  $\times$  red foxes produces a smoky red fox, where the colour of the red fox is strongly, but not completely, dominant. Crosses between Alaskan and standard foxes produce »blended cross foxes» with characters from both of the first mentioned hybrids. The genetics of these crosses is explained by WARWICK and HANSON (ASHBROOK, 1937) as follows:  $AABB$  red fox,  $aaBB$  Alaskan black fox,  $AAbb$  standard fox,  $aABB$  cross fox,  $AABb$  smoky red hybrids,  $AaBb$  blended cross foxes, etc. Breeding data seem to fit in very well with this hypothesis.

The modern *silver fox* is a product of selection from the original, almost wholly black, mutants. The silvering is caused by an intermingling of light banded guard hairs among the black hairs. The wild red fox has such silver hairs in varying numbers, particularly on the rump and loin, and some silver hairs are found even on the most genuine black foxes. The silvering is a quantitative character which is present in both red and black foxes, but the black fox and its mutants, e.g. white face and platinum, which will be discussed later, are qualitatively different from one another and from the red fox.

The common red fox, which ranges over the northern parts of

America, Europe and Asia, ought to be considered as belonging to one and the same species, *Vulpes vulpes* L., as pointed out by COLE and SHACKELFORD (1943). Local varieties or breeds can easily be distinguished, but, owing to the fact that all these varieties freely interbreed as soon as opportunity offers, there seems to be no logical reason to speak of different species. Mutant black foxes have also appeared from time to time among the wild living Scandinavian red foxes, and some of these mutants have been kept in captivity long before any importations of North American silver foxes were made. Such mutants have not been utilized, however, to build up any strains of our present day farm foxes. Sometimes the breeders may use Scandinavian red foxes for crossing with the farm-raised silver foxes, and in this way some of the local »*vulpes* blood» may find its way into the breeding stock. The Scandinavian silver fox breeding is, however, almost exclusively founded on imported animals, viz. the Canadian standard silver fox, probably due to the fact that skilled fox farming with selection for high quality pelts and extensive silvering started much earlier in North America than in the Scandinavian countries.

In Canada and Northern U. S. wild living black foxes and their cross products with the red fox seem to be of rather frequent occurrence, but in Sweden they are exceptional. BUTLER (1945) has studied the data on more than 700,000 Canadian fox pelts of the Hudson's Bay Company fur returns 1915—1944. He found the highest percentage of silver fox pelts in British Columbia (8,0 % silver and 41,3 % hybrids) and the lowest in Saskatchewan (3,0 % silver and 24,6 % hybrids). The frequency of silver foxes has decreased during the last 30 years from about 9 to 3 per cent. Three possible explanations of this decrease are put forward, viz. migration, differential survival or reproductive rates, and changed classification standards. WARWICK and HANSON's factorial hypothesis fitted well to the observed frequencies of the different colour phases.

## NEW MUTANT COLOUR PHASES OF THE SILVER FOX.

### A REVIEW OF THE LITERATURE.

Several new colour phases have appeared among farm bred silver foxes, and three such mutants are well described in the literature, viz. the white face, platinum and pearl platinum fox. The last-mentioned will be referred to here simply as the pearl fox, because it has nothing to do with the platinum character, and the term pearl platinum is apt to cause

confusion (BOWNESS, 1944). The platinum fox is in the U. S. usually called »platinum silver» or »Norwegian platinum», in Canada »standard platinum», and in the Scandinavian countries »Mons-platinum» — after the original mutant — or simply platinum. The latter term would seem to be adequate. Different names are also used for the white face fox, e.g. »white-marked silver», »ring-neck» or »platinum type silver», while in Norway it is spoken of as »Hovbrender platinum». Any name of the white face fox, which involves the term »platinum» should, however, be avoided.

The platinum fox is well described by MOHR and TUFF (1939) and by COLE and SHACKELFORD (1943). The latter authors have also fully described the white face fox. A brief characterisation here of these mutants will, therefore, be sufficient.

The *white face fox* is very similar to the common silver fox, except that it has more or less extensive white markings on the nose, forehead, neck, feet, breast and belly; the tip of the tail is white to the same extent as in the silver fox. The pigmentation of the underfur, the guard hairs and the ears, trunk and tail has about the same intensity as in the silver fox. There is no sharp borderline between the silver and the white face fox; the former may have white markings on the feet, breast and belly, and the latter may lack the white blaze along the nose, and the white collar (Fig. 1). There is the possibility, therefore, that the classification of the two types may sometimes be wrong. The variation in markings and shade of colour is great, and there may be some overlapping at the other end of the scale, towards the platinum fox. The light white face fox often shows a kind of roaning, owing to the presence of numerous wholly white guard hairs among the banded silver hairs. These white hairs give to the fur a »dead» appearance, and the lustre of the fur will not be the same as in the true silver fox. COLE and SHACKELFORD describe the white face foxes as »silvers on which the characteristic white-face markings have been superimposed».

The white face mutation seems to have occurred independently a number of times in U. S. A., Canada and Scandinavia. COLE and SHACKELFORD speak of the Colpitts, McNeil, Garvey, Holman and Spring-born strains of white face, some of which have probably descended from separate mutations. The Colpitts strain seems to be the oldest one; it is said to have originated in 1928. The »Hovbrender platinum» (MOHR and TUFF, 1939) is nothing else but a white face fox which has originated in Norway. COLE and SHACKELFORD give some figures from crosses between two American white face strains, designated A and B,

which tend to show that these strains are genetically different, this being due to mutations in different loci, and perhaps different chromo-

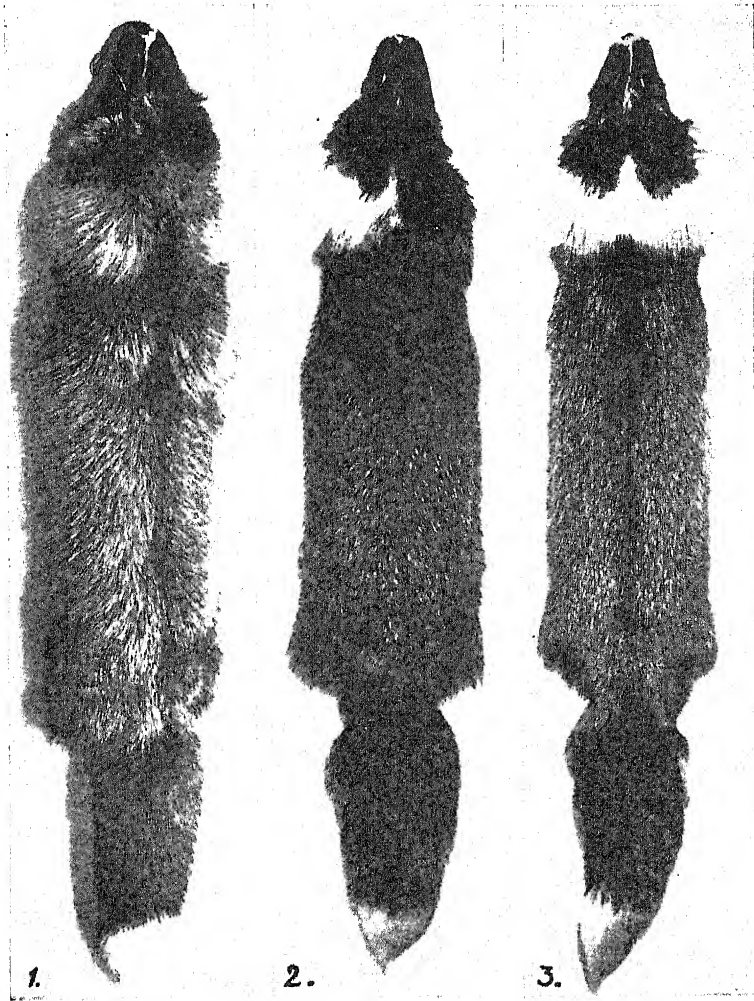


Fig. 1. Variations in the white markings of the white face fox. — To the left (1) a pelt which may be classified as silver, but it is really a white face with a small blaze and white markings on the feet and underline. — In the middle (2) a very heavily pigmented white face pelt with narrow blaze and broken collar. — To the right (3) a typical white face pelt with a narrow blaze, fairly broad and symmetrical collar, and the same intensity of pigmentation along the back and tail as in the silver fox.

somes. When the two strains are crossed with one another, the offspring shows more extensive white markings than either of the parent

strains, the white extending up on the middle of the ear while this is never the case in the common white face.



Fig. 2. Fox whelps of typical white face (above) and platinum (below) character.

The white face foxes on Swedish fox farms derive from Canada, perhaps mostly from the McNeil strain, and from Norway. The writer does not know of any original mutations in Sweden, and all our white face foxes would seem to be identical as far as the genotype for this particular character is concerned.

The *platinum fox* has a dilute pigmentation. Its underfur is distinctly lighter than that of the silver and white face foxes, the colour ranging from white to greyish; many guard hairs are entirely unpigmented, and others are pigmented only below the tapered end. The white blaze, the white collar around the neck, and the white markings on the feet, breast and belly are usually much more extensive than in white face foxes, and the ears are much lighter coloured; the blaze is as a rule narrow in a white face fox but broad in a platinum (Fig. 2). There cannot be any mistakes in the classification of platinum and silver foxes, but the variation of platinum and white face may, to some extent, overlap. By the lighter ears and underfur and the broader blaze it should be possible, however, to distinguish the true platinum fox. COLE and SHACKELFORD (1943) point out that it is possible that some of this variability is non-genetic, but they state that »it is more probable that much of it is determined by factors in the silver foxes to which the platinum foxes are bred». The usual variation in the pigmentation of Swedish platinum foxes is shown in Fig. 3.

The first known platinum mutant appeared in Troms in Northern Norway, where the famous platinum male »Mons» was born in 1933 (MOHR and TUFF, 1939). According to COLE and SHACKELFORD (1943), at least three independent platinum mutations have occurred in the U. S. and Canada, originating the Corbin, the Cody and the La Forrest strains. All these four platinum strains are phenotypically very similar, and there is no evidence so far to show that they are genetically different. The platinum foxes which are found in Swedish fox farms originated, as far as the writer is aware, from Norway. We have no records to show that independent platinum mutations have arisen in Sweden, whereas in Norway several platinum mutations seem to have occurred.

Original data on the inheritance of the white face and platinum characters have been published by MOHR and TUFF (1939), COLE and SHACKELFORD (1943) and GUNN (1945). MOHR and TUFF concluded that the platinum character is inherited as an autosomal dominant. They suggested that the mutant gene is lethal in a homozygous condition, but the data in support of this suggestion were admittedly very meagre. COLE and SHACKELFORD reached the conclusion, however, that the suggestion as to the lethality of the platinum gene was correct, and that the mutant white-face gene behaves in exactly the same manner as that of the platinum. They suggested that the genes for silver, white face and platinum are allelic to one another, any of the possible

three combinations of these mutant genes being lethal. Completely white whelps with blue eyes were occasionally found in the newly born litters after matings of mutant  $\times$  mutant, and the authors assume that these whelps represent the homozygous dominant type, which is

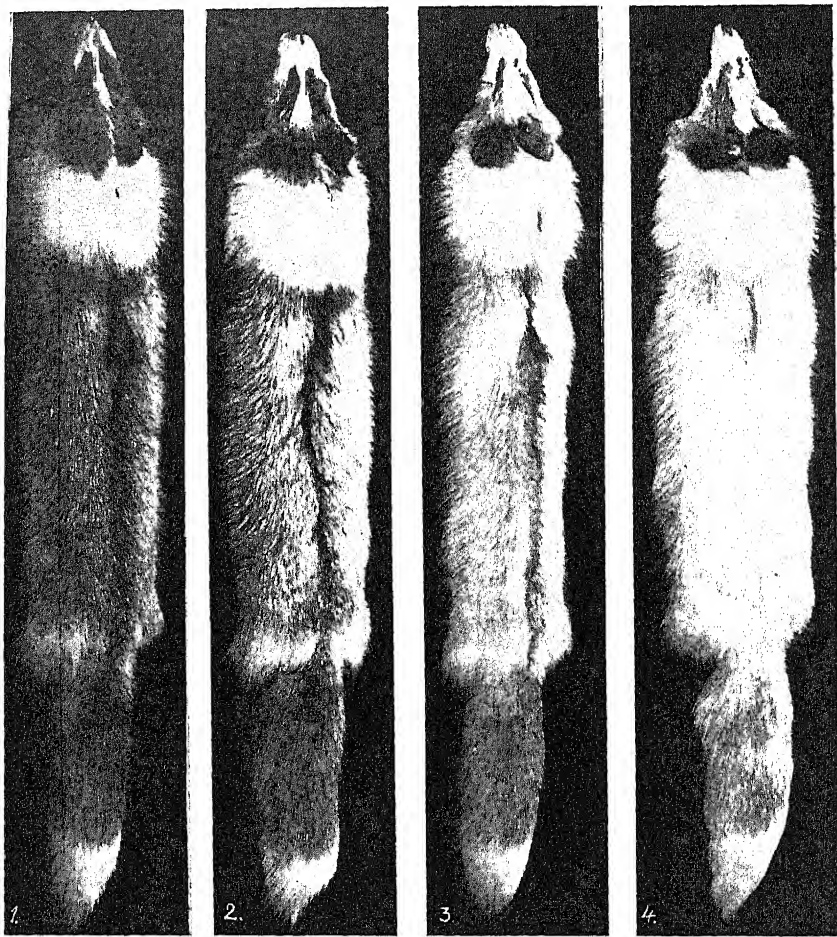


Fig. 3. Variations in the intensity of pigmentation of Swedish platinum foxes. Darker platinum pelts than the darkest one above (1) may be found.

not viable. One such white whelp lived, however, to about five weeks of age. Three white whelps after matings of platinum  $\times$  platinum, listed in Table 1, were all borne by the same vixen and sired by the same male. The litter size in COLE and SHACKELFORD's matings of white face  $\times$  platinum is very high and does not lend support to the hypo-

thesis that the genes for these two characters are allelic with lethal effect when combined in the same zygote. Only one critical »test mating« is referred to: a platinum male, offspring from a white face  $\times$  platinum cross, was mated to 3 silver females and produced 9 platinum pups, 10 silvers and no white face, i.e. the proportions expected if the male did not carry the white face gene. Furthermore, the mutational changes are depicted diagrammatically as a progressive deficiency; one

TABLE 1. *Data from crosses between mutant foxes.*

Matings	Number of litters	Litter size	Type of offspring			
			Silver	Plati- num	White	White face
<i>Platinum × platinum:</i>						
MOHR and TUFF (1939) .....	2	3,5	2	5	—	—
COLE and SHACKELFORD (1943) ...	?	?	10	22	—	—
	11	2,1	8	12	3	—
Total			20	39	3	
<i>White face × white face:</i>						
MOHR and TUFF (1939) .....	3 <sup>1</sup>	4,0	4	—	—	8
COLE and SHACKELFORD (1943) ...	2 <sup>2</sup>	4,5	1	—	5	3
	79	3,3	79	—	—	183
GUNN (1945) .....	(141) <sup>3</sup>	3,5	115	—	—	238
Total			299	—	5	432
<i>White face × platinum:</i>						
COLE and SHACKELFORD (1943) ...	6	6,1	15	16	—	6
GUNN (1945) .....	(14) <sup>3</sup>	3,1	12	16	—	16
Total			27	32	—	22

<sup>1</sup> »Hovbrenderer platinum«. <sup>2</sup> Selected litters where white pups were produced.

<sup>3</sup> Number of matings.

break at the end of the chromosome causes the white face character, and a second break the platinum. A viable fox must have at least one of the homologous chromosomes intact. GUNN presents further evidence as to the non-viability of the homozygous mutants, but he does not assume that the genes for white face and platinum are allelic.

The data from crosses between the mutant types, on which the previous workers have based their conclusions, are summarized in Table 1.

The combined data from the three sources are still rather meagre, and the results from the white face  $\times$  white face and the white face  $\times$

platinum matings do not agree too well with the 1 : 2 and the 1 : 1 : 1 ratios, which are expected on the basis of the proposed hypothesis.

The third mutant is the *pearl fox*, which is recessive to silver, or black. The first animals of this type were reported from a farm in Minnesota, U. S. A., in 1934 (BOWNESS, 1944). The pearl fox has an even blue-greyish colour throughout and no white markings, except the white on the tip of the tail and the white bands on the silver hairs. Two genetically different strains are known, the Ontario and the New Brunswick strains (DEAKIN, 1942; GUNN, 1945), which when crossed with one another produce only silver foxes, heterozygous for both the pearl genes. Breeding within the same strain yields only pearl foxes. When pearl foxes are mated to white face foxes, 50 % white face and 50 % silver pups are obtained. These white face foxes carry the recessive gene for the pearl character, and when they are mated to homozygous pearl foxes 25 % silver, 25 % white face, 25 % pearl and 25 % *pearlatina* pups are obtained. The *pearlatina* is a pearl fox with white face markings. *Glacier blues* are produced in the same way by using platinum for the cross instead of white face (COLE, 1945). Glacier blues are dilute pearl foxes with platinum markings. As far as the writer knows, the pearl fox does not exist in the Scandinavian countries.

### OWN INVESTIGATIONS.

The present writer has made an effort to analyse the inheritance of the white face and platinum mutants. Two different lines of approach have been followed; statistical data, compiled from Swedish fox farms, were analysed, and in the breeding season of 1946 an experimental inquiry was made regarding the intra-uterine development of the pups of platinum parents. The statistical work may be considered as completed, but the experimental breeding will be continued next season, and probably will have to be continued for several years before the problem of what happens to the homozygous mutants is solved.

#### AN ANALYSIS OF BREEDING DATA FROM SWEDISH FOX FARMS.

At the end of each breeding season the members of the Swedish Fur Breeders' Association report all matings on their respective fox farms to the office of the association. About the first of August all whelps in the litters are ear marked by tattooing, and a report on the number of young born in each litter, and also the ear number, the colour phase, and the parents of each whelp which is alive at the date

TABLE 2. Reproductive results of different mating combinations.

M a t i n g s	Empty vixens		Abortions		Number of pups in the litter				Number of pups born in litters where a count was made		Mortality during the first three months		
	Total num-ber of mated vixens	Per cent of total vix-ens <sup>1</sup>	Num-ber of abor-tions	Per cent of preg-nant vix-ens <sup>1</sup>	Number of pups in the litter		Total pups	Aver-age litter size	Total pups dead	11	12		
					Not counted <sup>2</sup>	Counted							
												Number of litters	Per cent of total litters <sup>1</sup>
	1	2	3	4	5	6	7	8	9	10	11	12	
Silver X silver .....	6,280	223		56	0,92	156		2,60	5,845	26,165	4,48	2,536	9,09
Silver ♀ X white face ♂ .....	1,029	41		7	0,71	32		3,26	949	4,228	4,46	365	8,63
Silver ♀ X platinum ♂ .....	1,911	74		13	0,71	58		3,18	1,766	7,975	4,52	1,164	14,60
Silver ♂ X white face ♀ .....	671	58		28	4,57	49		8,38	536	2,358	4,40	330	13,99
Silver ♂ X platinum ♀ .....	201	20		10	5,52	14		8,19	157	667	4,25	154	23,09
White face X white face ..	286	25		11	4,21	26		10,40	224	808	3,61	158	19,55
White face ♀ X platinum ♂	159	20		1	0,72	9		6,52	129	473	3,67	61	12,90
White face ♂ X platinum ♀	27	3		1	4,17	1		4,35	22	71	3,23	(2)	(2,82)
Platinum X platinum .....	75	10		2	3,08	5		7,94	58	190	3,28	28	14,74

<sup>1</sup> The percentages are somewhat too low throughout owing to the omission of most of the vixens, which were culled because of breeding failures at one year of age. In comparing the different classes with one another the percentages may be supposed, however, to give a reliable indication of existing inter-class differences.

<sup>2</sup> All pups in the litters died early after birth.

of tattooing is sent to the office. The second report can be checked against the first one as to the parentage of the litters. The pups in the litters are usually counted within a few days after birth, but on some farms the count may be made as late as a week after parturition. Notes are not always made at the same time, however, on the type of pups in the litters. Therefore the data are incomplete as to the type of the pups which have died before they are tattooed. On the following pages the deaths between birth and tattooing are referred to as »mortality during the first three months after birth». This time interval of three months is, however, only an approximate average from which the age of an individual litter may deviate rather considerably.

The breeding data from the sources stated were transferred to mimeographed cards. One card was written out for each vixen, so that a card would give the whole reproductive history of the vixen. Most of the cards, and in every case those with data on matings of mutant  $\times$  mutant, were afterwards checked on the fox farms in order to avoid, as far as possible, omissions and errors. The result of the analysis of this material is presented in Tables 2, 3 and 4.

The most pertinent figures of Table 2 in relation to our present problem are those in col. 10 giving the average litter size from the different mating combinations. The summarized results are as follows:

Matings	Total litters	Average litter size	Standard deviation of the litter size
Silver $\times$ silver .....	5.845	$4.48 \pm 0.0182$	1.394
Silver $\times$ mutant .....	3.408	$4.47 \pm 0.0242$	1.411
Mutant $\times$ mutant.....	433	$3.56 \pm 0.0636$	1.323

The matings of silver  $\times$  silver and silver  $\times$  mutant have produced litters of practically identical average size, but after matings of mutant  $\times$  mutant the litters are reduced by 20.1 per cent of what may be considered their normal size. The difference in litter size between mutant  $\times$  mutant and the other two types of matings is highly significant. In Table 2 it is seen that the litter size from matings of silver  $\sigma \times$  mutant  $\phi$  is a little lower than from the reciprocal mating, mutant  $\sigma \times$  silver  $\phi$ , and it seems likely that the difference can be explained by the higher mortality in litters from vixens of the mutant types. In matings of mutant  $\times$  mutant the average litter size from white face vixens is  $3.63 \pm 0.0713$  but from platinum vixens only  $3.26 \pm 0.1353$ . The former average is higher, but the latter is a little

lower than would be expected if 25 per cent of the pups in litters from mutant  $\times$  mutant matings die in the uterus, or immediately after birth, and thus escape counting. The expectation under such circumstances would be  $4.48 \times 0.75 = 3.36$ . The difference between the two averages is  $0.37 \pm 0.1529$  or  $2.42$  times its standard error, and the probability of obtaining this result due to chance in sampling is less than 0.02.

In order to reveal, if possible, the causes of the differences in litter size of the various mating combinations, an account was made of all mated vixens and all the litters; the figures obtained are presented in Table 2. In cases where all the pups in a litter had died early after birth they were not always counted, or notes of the count were not made. This fault, as well as the differences between groups in the frequency of »empty vixens» and abortions, may affect the average litter size of the different groups. The figures presented (columns 3, 5, 7 and 12 of Table 2) do not, however, show a higher fetal or post-natal mortality after mutant  $\times$  mutant matings when the mother of the litter is a platinum than when she is a white face fox. It might be assumed that the homozygous white face pups are viable in some cases, whereas the homozygous platinum are always non-viable, but this does not explain the fact that the greatest difference in litter size is found between the two reciprocal matings white face  $\times$  platinum, where the pups would be supposed to be, on an average, genetically identical. Therefore, it seems most logical to assume that the actual figures for the average litter size in the four different matings of mutant  $\times$  mutant are all approximations of one and the same theoretical value, i.e. 75 % of the normal litter size in matings of silver  $\times$  silver or silver  $\times$  mutant, the deviations being due to chance in sampling, or to differential mortality in the litters.

In Table 3 data are presented showing the type-segregation of the offspring from different matings of mutant  $\times$  silver and mutant  $\times$  mutant. The obtained ratios are compared to those expected according to COLE and SHACKELFORD's hypothesis that the hereditary factors for the silver, white face and platinum characters form a series of multiple alleles, here denoted  $W^+$ ,  $W$  and  $W^P$ , and that all the three possible combinations of two of the mutant genes in one individual, i.e.  $WW$ ,  $WW^P$  and  $W^PW^P$ , are lethal. The significance of the deviation of obtained from expected frequencies is tested by PEARSON's  $\chi^2$  test, and the P value corresponding to each computed  $\chi^2$  is given in the table (FISHER, 1932). There is a good agreement between the obtained and the expected frequencies except in the silver  $\times$  platinum matings,

TABLE 3. *Mendelian segregation in the offspring from different mating combinations, and early mortality of silver, white face and platinum pups.*

Mating s	Total number of pups of known type				Mortality during the first three months after birth									
	Actual numbers				Ratios				Number of pups dead before three months of age				Percent of total pups of known type	
	Sil-ver = S	White face = W	Plati-num = P	Obtained	Expected	P <sup>1</sup>	Type unknown		Type known			Sil-ver	White face	Plati-num
							Num-ber of pups	Per-cent of total pups born	Sil-ver	White face	Plati-num			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Silver × platinum.....	4157	—	3842	1S: 0.93P	1S: 1P	0.0001 0.15 <sup>2</sup>	556	6.5	258	—	504	6.2	—	13.1
Silver × white face .....	3038	2986	—	1S: 0.98W	1S: 1W	0.50	256	4.1	201	238	—	6.6	8.0	—
White face × white face	267	483	—	1S: 1.81W	1S: 2W	0.21	22	2.9	54	82	—	20.2	17.0	—
White face × platinum.	167	188	182	1S: 1.13W: 1.00P	1S: 1W: 1P	0.50	9	2.5	14	18	22	8.4	9.6	12.1
Platinum × platinum ...	58	—	127	1S: 2.10P	1S: 2P	0.85	5	2.6	4	—	19	6.9	—	15.0
Total and averages	7687	3657	4151	—	—	—	848	5.2	531	338	545	6.9	9.2	13.1

<sup>1</sup> Probability that the deviation between the obtained and the expected ratios may be due to chance in sampling (Pearson's  $\chi^2$  test).

<sup>2</sup> P is calculated on the assumption that, of 556 pups of unknown type, 66.14 % were platinum, as is the case among the dead pups of known type.

where a highly significant discrepancy is noted. We think, however, that this discrepancy can be explained on the basis of the following two facts: (1) the frequency of dead pups of unknown type is very much higher in this class of matings than in any of the other four classes, and (2) the mortality of the platinum pups is almost twice as high as that of the silvers. The rather pronounced difference between the classes in percentage pups of unknown type is probably due to the data not having been random samples. The mating silver ♀  $\times$  platinum ♂ is used most extensively, by skilled and unskilled fox farmers, but the mating of mutant  $\times$  mutant has been practised almost exclusively by skilled breeders, who keep more accurate and complete records than their less skilled colleagues. The mating silver ♀  $\times$  white face ♂ is also widely practised, but here the difference in death-rate between the two classes of pups is comparatively small.

If we assume the proportion of silvers to platinums among the 556 pups of unknown type which perished early from the silver  $\times$  platinum matings to be the same as that of the pups of known type from the same mating, i.e. 258 silvers : 504 platinums, or 66.14 % platinums, then we would classify 188 pups of the unknown type as silvers and 358 as platinums. The obtained ratio would then be  $(4157 + 188) : (3842 + 368)$ , or 1 silver : 0.97 platinum, and the P value would rise to 0.15. Under this supposition the obtained ratios in all mating combinations are in good agreement with the expected ratios.

It seems likely that the agreement between the obtained and the expected frequencies would have been still better if the pups had been counted and classified in all litters immediately after birth. Table 2 presents the frequency of litters where no count, or classification, of the pups was made. Because of the excessive mortality of platinum pups it may be expected that their proportion in these lost litters was relatively high. Furthermore, as the count and classification are often postponed to several days after the birth of the litter, it may be expected that a number of pups die and are eaten by the mothers before any notes of their existence are made. In order to obtain the exact frequencies of the pups in all classes, the vixens must be carefully watched at the time of parturition, and immediate counts and classifications must be made.

The writer has tried to test the previously mentioned hypothesis also by a search for such foxes from mutant  $\times$  mutant matings as have been used for breeding and have produced offspring. The breeding history of such foxes was investigated, and the following results obtained.

(1) Eleven platinum foxes from platinum parents were found. Six of these foxes were mated to silvers and produced in 20 litters, 41 silver and 32 platinum pups. All six foxes produced silver pups and were accordingly heterozygous for the platinum character. The other 5 platinum foxes were mated to platinum and white face. Three of these foxes threw silver pups and a fourth a white face, and consequently they were also heterozygous, but one after mating to platinum produced only 4 platinum pups, and this fox might have been homozygous. On a chance basis we would expect, however, to obtain such a result in one trial out of five when heterozygous platinums are mated to one another.

(2) Eight white face foxes from white face parents were mated to silvers and produced in 8 litters, 18 silver and 17 white face pups. One of the eight litters contained only silver and one only white face pups. Three white face foxes from white face parents were mated to white face and produced in each one of 3 litters both silver and white face pups. Of these 11 white face foxes from white face parents 10 were certainly, and one probably, heterozygous for the white face character.

(3) Three platinum and two white face foxes from platinum  $\times$  white face matings were found. Their breeding results are presented in Table 4.

TABLE 4. *Breeding tests of offspring from matings of platinum  $\times$  white face.*

Tested animals	Mated to	Total litters	Number and type of pups			
			Total	Silver	White face	Platinum
Platinum D 202 ♂ ...	9 silver ♀♀	9	39	26	—	13
» B 143 ♀ ...	3 silver ♂♂	3	17	8	—	9
» H 62 ♂ ...	2 silver ♀♀	2	8	6	—	2
White face D 15 ♀ ...	1 silver ♂	1	3	2	1	—
» D 15 ♀ ...	1 white face ♂	1	3	1	2	—
» B 27 ♀ ...	2 white face ♂♂	2	9	3	6	—

Homozygous platinum foxes have never been found by previous workers, and they are not found in our material. As to the white face, KELLOGG (1946) states: »In other herds it has been reported that a certain strain of white marked animals when mated repeatedly to silver foxes produce litters with 100 per cent white marked progeny. This

would seem to be sufficient evidence that the homozygous white marked fox can be produced». In the U. S., where COLE and SHACKELFORD's (1943) data indicate that two genetically different strains of white foxes may occur, it would be possible that the homozygous mutants are non-viable in only one of these two strains. In Sweden we have no records of homozygous white face foxes, and our own data point rather decisively towards a general lethality of this gene combination. All available facts fit in with the hypothesis on the allelic nature of the white face and platinum genes. A crucial test of this hypothesis is to breed platinum offspring from platinum  $\times$  white face matings with silver foxes. If the genes for the platinum and white face characters are allelic, and their combination in the same zygote is lethal, any platinum fox of the stated parentage should, mated to silvers, produce only silver and platinum, but never white face offspring. Furthermore, any white face fox with the parentage white face  $\times$  platinum should, when mated to silver or white face, produce only these two types of offspring and never platinum. In Table 4 three platinum and two white face foxes obtained from platinum  $\times$  white face matings are thus tested. The number of tested animals is, of course, too small to allow definite conclusions, but as far as the breeding results go, they point towards the allelic gene hypothesis <sup>1</sup>.

Our data show quite conclusively that the heterozygous white face and platinum foxes are less viable than the silver fox, and that the reduction in viability is progressive in exactly the same way as the mutational change in the pigmentation of the animals:  $W^+ \rightarrow W \rightarrow W^P$ . The pup mortality during the first three months after birth is for silver 6,91 %, white face 9,24 % and platinum 13,13 % (Table 3), and the frequency of failures in reproduction is much higher for the mutant vixens than for silvers (Table 2). On an average the reproductive efficiency is lower for the platinum than for the white face vixens. The fox breeders know this by experience, and therefore mutant vixens are used for breeding much less frequently than mutant males. Some

<sup>1</sup> Since the galley proof of this paper was read the writer came across a statement by C. K. GUNN [Fur Trade Journal of Canada, 1946, 23 (6): 12] that he had on record 17 cases of matings platinum  $\times$  silver foxes where typical white marked pups had appeared in the litters. On the assumption by COLE and SHACKELFORD (1943), referred to on p. 155, that two non-allelic white face mutants exist in North America, the observations reported by GUNN are not surprising, and they do not interfere with the conclusion that one of these mutants is allelic to the gene for the platinum character.

breeders have mated white face  $\times$  white face and platinum  $\times$  platinum in the hope of obtaining homozygous animals of the mutant types. This effort is wasted, however, and owing to the frequent breeding failures after such matings, as well as small litters and high pup mortality, the economic aspects are not promising.

#### EXPERIMENTAL MATINGS PLATINUM $\times$ PLATINUM.

Through the kind co-operation of the fox breeders it was possible for the writer to obtain five one-year old platinum vixens for breeding experiments in the season of 1946. The vixens were placed on the experimental farm of the Swedish Fur Breeders' Association, where housing, feed and care were provided free of charge. The plan was to mate all the five vixens to platinum males, and to try to find out what happened to the homozygous platinum pups. On the basis of COLE and SHACKELFORD's (1943) observations, referred to on page 158, we assumed that the genetic lethality became effective rather late during the fetal development, and it was decided to kill the vixens, at least those that were mated early in the season, after 45 days of pregnancy. At this stage it should be possible to ascertain the number and type of living fetuses in the uterus, and also the number of dead and perhaps partly resorbed ones.

Unfortunately only three of the vixens mated and became pregnant. One of the empty vixens (H 199) came on heat so late in the season that no active males were available, and as to the other one (H 230), intromission was not possible in spite of vigorous attempts by several males. The vixen H 199 was sent back to the breeder, and H 230 was killed 10 days after the termination of oestrus.

Immediately after being killed the vixens were opened and their reproductive organs examined. An intersection was made in the uterine wall for each locus of fetal attachment, and the type and state of development of the fetuses were determined. Then the uterus and its content, with the placental relations between fetuses and uterus intact, were fixed in 10 % formalin for later investigations in the laboratory.

When the uteri were opened, it was found that all fetuses were living and in the same state of development. A very careful search was made later, on the formalin-fixed material, for partly resorbed fetuses, and for sites of implantation of resorbed fetuses, but nothing of this kind was found. The type of each fetus could easily be determined beyond doubt. Fig. 4 shows the three litters, photographed after the formalin fixation. The ovaries of the four vixens were sectioned, and

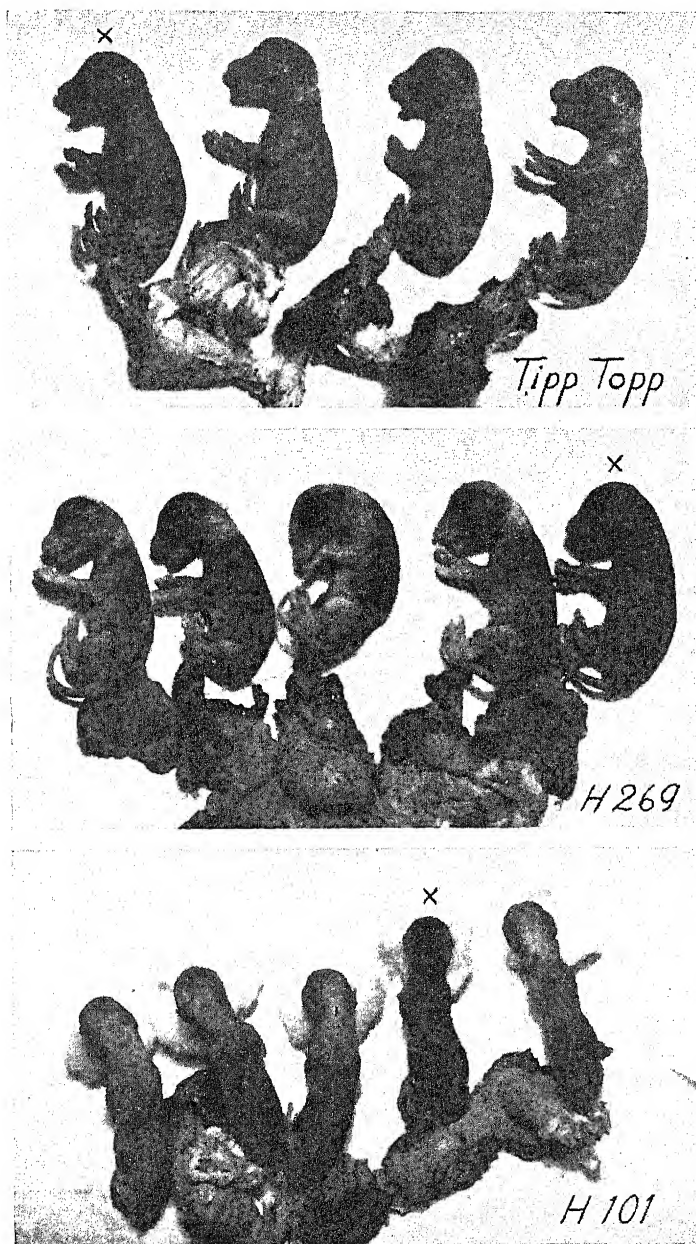


Fig. 4. Fetal litters from the breeding of platinum  $\times$  platinum. The silver pups are marked with an  $\times$ .

TABLE 5. *Result of breeding platinum vixens to platinum males.*

Experimental vixen	Date of mating	Date of killing	Pregnant days	Number of living fetuses in uterus			Number of corpora lutea in the ovaries
				Total	Silver	Platinum	
Tipp-Topp	Febr. 28th	April 13th	44	4	1	3	5
H 269	March 2nd	April 16th	45	5	1	4	5
H 101	March 16th	April 30th	45	5	1	4	5
		Total	—	14	3	11	15
H 230	On heat March 1st and 2nd	March 11th	—	—	—	—	5

the corpora lutea counted in order to ascertain the number of ova shed during oestrus. The pertinent details are given in Table 5.

The uteri of the three vixens contained 14 fetuses — 11 platimums and 3 silvers — and the total number of corpora lutea in their ovaries was 15. There were no white or only slightly pigmented fetuses, and no evidence whatever of any intra-uterine mortality. Because of (1) the normal size of all three litters and (2) the close correspondence between the number of corpora lutea and the number of fetuses, it is not probable that there had ever been any selective intra-uterine mortality. At most one zygote could have perished early because only 15 ova were shed at the time of oestrus.

The intensity of pigmentation of the platinum fetuses varied somewhat, as shown by Fig. 4, but the variation was continuous, and it was

TABLE 6. *Average weight of silver and platinum fetuses at the 45th day of intra-uterine development, and the absolute and relative weight of some of their internal organs.*

Type of fetuses	Number of fetuses	Average weight of fetus and organs								
		Whole fetus	Lungs	Alimentary tract	Liver	Heart	Kidneys	Spleen	Thymus	
Silver	3	54,68	Weight in grams	2,30	2,47	4,31	1,23	0,72	0,12	0,18
			% of total weight	4,21	4,52	7,88	2,25	1,32	0,22	0,33
Platinum	11	55,44	Weight in grams	2,43	2,71	3,98	1,19	0,71	0,12	0,17
			% of total weight	4,38	4,89	7,18	2,15	1,28	0,22	0,31

not possible to classify any fetus as being presumably a homozygous platinum. All the 14 fetuses were weighed and dissected, and a careful search was made for anatomical abnormalities, but none was found; some of the internal organs were also weighed. We present here only the average weights of the silver and platinum fetuses. The variation within the two groups was continuous.

The differences between the averages of the two groups are not significant. No objective measures of the quantity or quality of the blood were taken, but when the formalin-fixed fetuses were cut through in the median line, after evisceration, there was a consistent and very striking difference between silver and platinum fetuses in the intensity of blood pigmentation. The platinums appeared pale and anaemic in comparison with the silvers. That anaemia is common in mutant foxes, particularly in the platinums, has been pointed out by several writers, e.g. GUNN (1946). According to TODD (1944) 10—35 % of the platinum pups are lost (U. S. A. and Canada) during the first weeks after birth, and he states that the major cause is internal hemorrhage due to lack of vitamin K. The hemorrhage is said to be more frequent among the very light platinums than among the darker ones. Also, the mature platinum foxes show, according to TODD, an increasing tendency to become anaemic with decreasing intensity of pigmentation.

The present writer will make an attempt to study the blood picture of mutant foxes in connection with future breeding experiments.

It may be taken for granted that the platinum  $\times$  platinum cross is a case of monohybrid inheritance, where the mutant gene has a lethal effect in the homozygous condition. The segregation of 11 platinum : 3 silver fetuses in our breeding experiment (Table 5) may, however, be explained in three different ways. The three hypotheses are as follows.

(1) The platinum gene ( $W^P$ ) is completely dominant over its normal allele ( $W^+$ ) as far as its effect on the pigmentation is concerned. All fetuses are viable during the intra-uterine development, but the homozygous pups ( $W^P W^P$ ) are non-viable after partus; as a rule they are eaten by the mother together with the placenta. According to this hypothesis the intra-uterine ratio would be 3 platinums : 1 silver, and the average number of fetuses per litter would be the same as in matings of silver  $\times$  silver or silver  $\times$  mutant. Post partum the ratio would be 2 platinums : 1 silver, and the litter average would be reduced to 75 % of its normal size.

(2) The lethality of the platinum gene in double dose is effective very early during the intra-uterine development and therefore no traces

of partly resorbed fetuses or sites of implantation are found. The intra- and extra-uterine ratio would be the same, i.e. 2 platinums : 1 silver.

(3) The homozygous platinum fetuses die late during pregnancy or a short time after birth. They are white in colour, and therefore it would be possible to classify them according to their genotype, as assumed by COLE and SHACKELFORD (1943). The ratio in uterus and at partus, when all fetuses, dead and living, are ascertained, would then be 1 homozygous white ( $W^P W^P$ ) : 2 heterozygous platinums ( $W^+ W^P$ ) : 1 homozygous silver ( $W^+ W^+$ ).

The probabilities (P) of obtaining 11 platinums + 3 silvers in a sample of 14 pups on the basis of any one of the three hypothetical ratios are as follows.

Hypothesis	Ratio	Probability *)	P	P-ratios
1	3 : 1	$364 (\frac{2}{4})^{11} (\frac{1}{4})^3 = 0,2402$		$\frac{0,2402}{0,00277} = 86,7$
2	2 : 1	$364 (\frac{2}{3})^{11} (\frac{1}{3})^3 = 0,1559$		$\frac{0,1559}{0,00277} = 56,3$
3	1 : 2 : 1	$0,2402 (\frac{2}{3})^{11} = 0,00277$		

\* The coefficient 364 is calculated by expansion of the binomial  $(p + q)^{14}$ .

Although our material is limited to 14 fetuses, the 1 : 2 : 1 ratio may be ruled out owing to rather poor agreement with the actual data. P of the  $\chi^2$  test is less than 0,01.

Unfortunately, the number of fetuses from our experimental vixens is too small to decide between the 3 : 1 and the 2 : 1 ratio. That no evidence of intra-uterine mortality was found is, however, in favour of the 3 : 1 ratio. In the next breeding season, therefore, the writer will concentrate his efforts on a test of the hypothesis (1) that the homozygous platinums die at the time of partus, and that they are phenotypically similar to the heterozygotes as far as the pigmentation is concerned.

White or almost white pups have occasionally been found also on Swedish fox farms after matings of platinum  $\times$  platinum or platinum  $\times$  white face foxes, but their frequency could hardly reach more than a few per cent of the total number of pups in such litters. It seems probable that these slightly pigmented pups represent extreme segregates in a continuous series of dark to light heterozygous platinums.

The writer wishes to express his sincere thanks to the Swedish Fox Breeders' Association for the provision of working facilities at the Association's fox farm, and for all the help so readily given whenever needed in connection with the compilation of statistical data. To Mr. A. BACKHOFF, who was in charge of the work at the fox farm, thanks are due for careful observation of the experimental animals and for conscientious record keeping. Last but not least the writer is greatly indebted to those fox breeders who donated five platinum vixens for experimentation, and to all the hundreds of breeders who have willingly taken the trouble to check and complete the data on our record cards. It was this aid that made our work possible.

### SUMMARY.

Investigations were carried out on the inheritance of the white face and platinum characters, which have arisen through mutations in the silver fox. Statistical data from Swedish fox farms are presented in Tables 2, 3 and 4, showing litter size, pup mortality and Mendelian segregation in the matings of silver  $\times$  mutant and mutant  $\times$  mutant foxes. Experimental matings of platinum  $\times$  platinum were also carried out with the object of discovering the fate of the homozygous platinum fetuses (Table 5). The results are summarized in the following paragraphs:

(1) The normal litter size in matings of silver  $\times$  silver or silver  $\times$  mutant was found to be 4.48, but in matings of mutant  $\times$  mutant in any possible combination the litter size was reduced to 3.56.

(2) The segregation in matings of silver  $\times$  mutant and mutant  $\times$  mutant foxes is explained by COLE and SHACKELFORD's hypothesis that the hereditary factors for the silver, white face and platinum characters form a series of multiple alleles ( $W^+$ ,  $W$  and  $W^P$ ), and that all the three possible combinations of the mutant genes ( $WW$ ,  $WW^P$  and  $W^PW^P$ ) are lethal. When the differential mortality of the pups is taken into consideration our actual data fit in very well with this hypothesis.

(3) The heterozygous white face and platinum foxes are less viable than the silver fox, and the reduction in viability is progressive in the same way as the mutational change in the pigmentation. The pup mortality during the first three months after birth was for silver 6.91 %, white face 9.24 % and for platinum 13.13 %. The frequency of reproductive failures of the vixens follows the same general trend.

(4) In the experimental matings of platinum  $\times$  platinum three

vixens were killed after 45 days of pregnancy. Their uteri contained 14 fetuses — 11 platinum and 3 silver — and the corresponding number of corpora lutea in the ovaries was 15. All the fetuses were living when the vixens were opened, and no evidence of any intra-uterine mortality was found. All the platinum fetuses were phenotypically alike.

(5) The results of the breeding experiment can best be explained on the basis of a 3 : 1 ratio, assuming that the homozygous mutants are viable in the uterus but die immediately after partus and are, as a rule, eaten by the mother, together with the placenta. The 2 : 1 ratio, assuming that the homozygous mutants die very early during the fetal development, cannot be rejected, however. Further experiments are needed for a decision between these two hypotheses.

Uppsala, August 22nd, 1946.

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# SEX LINKAGE AND SEX DETERMINATION IN A MOSQUITO, *CULEX MOLESTUS*

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IN most animals so far studied in which sex is genetically determined there is a chromosomal difference between the sexes. One sex possesses two homologous sets of chromosomes. The other sex has one such complete set, and another set in which one or more members are absent or replaced by chromosomes of a different type. In some groups the male has only a haploid chromosome set. There are however a number of groups in which sex is wholly or mainly determined genetically, but in which no chromosomal differences between the sexes have been seen. These include all vertebrates except mammals and birds. In the *Nematocera*, the primitive suborder of the *Diptera* to which *Culex* belongs, some families have sex chromosomes, while others have not. According to the review of WOLF (1941), sex chromosomes are found in the *Phryneidae*, *Thaumaleidae*, *Scatopsidae*, and *Fungivoridae*, while no difference between the sexes has been found in *Bibionidae*, *Itoninidae*, *Culicidae*, *Melusinidae*, *Tendipedidae*, *Tipulidae*, *Limoniidae*, and *Cylindrotomidae*. In addition sex chromosomes are unknown in the *Chironomidae*. In the *Sciaridae* and *Cecidomyiidae* the somatic nuclei of the sexes differ, but their germ lines do not, so that the chromosomal difference is concerned with sex development rather than determination (v. WHITE, 1945).

It has often been thought that where no sexual difference has yet been found, a further study would reveal it. However no structural differences have been discovered in the large chromosomes of the *Urodela*, or in the giant polytene chromosomes of the salivary and other glands of the *Culicidae* and *Chironomidae* (BAUER, 1935, 1936; PHILIP, 1942; SUTTON, 1942). The last author examined the giant chromosomes of the Malpighian tubules of *Culex pipiens*.

Most genetical work has been done on animals with two *X* chromosomes in one sex, and an *X* and a *Y* in the other; and there has been a tendency to regard the results obtained in them, and particularly in

*Drosophila* species, as of universal validity. In this communication we present facts which are best explained on the hypothesis that in *Culex molestus* sex is determined by a single pair of genes, maleness being dominant over femaleness, and that another gene pair located in the same chromosome shows linkage of the usual type, that is to say partial coupling or repulsion, with them.

*Systematic position and origin of stock.* — White-eyed individuals appeared in a stock of *Culex* (*Culex*) *molestus* FORSSKÅL, which is very closely allied to the commoner *Culex* (*Culex*) *pipiens* L. It was originally regarded as a biological race of *C. pipiens* in which the adults do not hibernate, the females lay fertile eggs without a blood meal, and pairing can take place in a small space. However MARSHALL and STALEY (1937) give reasons for regarding this autogenous race as a separate species, a nomenclature now generally adopted. Among other differences, *C. molestus* bites human beings far more readily than *C. pipiens*. The subject has been fully reviewed by MARSHALL (1938). It must be remembered that *C. molestus* and *C. pipiens* give fertile hybrids (TATE and VINCENT, 1936).

Our stock of *C. molestus* originated from larvae collected by Mr. P. G. SHUTE, malaria officer, Ministry of Health, from a platform sump at Old Street Underground Station, London. The stock has been maintained under laboratory conditions since March 1944 by Dr. A. BISHOP, Molteno Institute, Cambridge, whom we have to thank for supplying us with egg rafts from this stock.

In a cage containing several hundred mosquitoes 4 white-eyed females and 10 white-eyed males were found, and all other white-eyed individuals were descended from them. The eye pigment is completely lacking, and no differences in other organs or in the shape or size of the eye have been noticed. The white eye can be seen in the first larval instar and in all later stages, however the numbers in Table 1 are counts of imagines.

*Methods.* — The experimental work was entirely carried out by GILCHRIST in the Department of Entomology of the London School of Hygiene and Tropical Medicine, HALDANE being responsible only for planning and interpretation. The mosquitoes were bred in a constant temperature room at 24° C—25° C, at a relative humidity of 70 %—80 %. In these conditions the life cycle was completed in 18 to 21 days.

Larvae were reared in enamel bowls containing tap water into which food, consisting of powdered dog biscuits and stabilized wheat

embryo (»Bemax») was sprinkled. The water was changed daily and fresh food added. The first pupae appeared 10 to 12 days after the eggs hatched, and all larvae pupated within the next 3 days. Pupae were removed daily, and put into a  $3 \times 1$  inch specimen tube. At this stage the normal and white-eyed individuals were separated, and the sexes were separated to a large extent. This was possible since males pupated about 24 hours earlier than females, so virgins could be obtained without isolating each individual. Up to 30 pupae could be placed in one tube without interfering with emergence. The tubes were plugged with cotton wool, and a strip of paper inserted into each which was held in place by the plug, and on which the newly hatched imagines could settle.

Paired matings were made in similar specimen tubes not more than 3 days after females had emerged. On the day after mating 1—2 cm. of water was run into the tube, and eggs were generally laid in the next 4 days. The number of eggs in a raft was counted, and it was transferred to a bowl, where the eggs hatched in 36 to 48 hours. The pre-imaginal mortality varied from about 5 % to 15 %, except in two series where it rose over 20 % owing, we have reason to believe, to accidental contamination with »D. D. T.» in dust.

*Sex ratio and single factor ratios.* — Counts of the flies derived from three rafts laid by the original white-eyed females with unknown mates suggested the form of sex linkage which was afterwards found, and systematic paired matings, with some mass matings, were undertaken. The results of all matings in which both parents were known are given in Table 1. Expectations are given in italics where segregation occurred.  $w$  symbolizes the recessive gene for white eye, and  $+$  its normal allelomorph;  $M$  symbolizes the dominant gene for maleness, and  $m$  its allelomorph. 36 of the 132 rafts were derived from matings of several females with one or more males. The remainder were from paired matings. The mean number of eggs per raft was 86.3. The means for normal and white-eyed mothers are 86.<sub>38</sub> and 86.<sub>27</sub> respectively, a very close equality.

The first two lines refer to stocks in which brother—sister mating was carried on for three generations in each case. The next two lines show the results of reciprocal crosses between them. It will be noted that the pre-imaginal mortality does not differ significantly in the three genotypes  $\frac{+}{+}$ ,  $\frac{+}{w}$ , and  $\frac{w}{w}$ . The remainder of the flies recorded in this

TABLE 1.

Mother	Father	Rafts	Eggs	Imagines	Normal ♀	White ♀	Normal ♂	White ♂	Remarks
$\frac{m+}{m+}$	$\frac{m+}{M+}$	9	702	636	310	0	326	0	
$\frac{mw}{mw}$	$\frac{mw}{Mw}$	9	728	646	0	308	0	338	
$\frac{m+}{m+}$	$\frac{mw}{Mw}$	2	137	124	61	0	63	0	Normal parent from inbred line.
$\frac{mw}{mw}$	$\frac{m+}{M+}$	2	138	118	53	0	65	0	
$\frac{mw}{mw}$	$\frac{m+}{M+}$	1	73	64	36	0	28	0	Parents from $F_2$ with white grandmother.
$\frac{mw}{m+}$	$\frac{mw}{Mw}$	4	297	262	69 (72)	75 (72)	64 (59)	54 (59)	Mother's mother white.
$\frac{mw}{m+}$	$\frac{mw}{Mw}$	2	147	145	46 (40)	34 (40)	33 (32.5)	32 (32.5)	Parents from $F_2$ with white grandmother.
$\frac{m+}{mw}$	$\frac{mw}{Mw}$	13	1222	901	233 (226)	219 (226)	227 (223.5)	220 (223.5)	Mother's father white.
$\frac{mw}{mw}$	$\frac{mw}{M+}$	24	2104	1623	41 (47.1)	711 (704.9)	824 (816.5)	47 (54.5)	Father's mother white.
$\frac{mw}{mw}$	$\frac{mw}{M+}$	7	548	491	14 (17.0)	257 (254.0)	209 (206.2)	11 (13.8)	Parents from $F_2$ with white grandmother.
$\frac{mw}{mw}$	$\frac{m+}{Mw}$	10	981	854	409 (411.5)	30 (27.5)	39 (26.0)	376 (389.0)	Father's father white.
$\frac{m+}{mw}$	$\frac{m+}{Mw}$	1	86	79	36 (36.6)	3 (2.4)	0 (2.5)	40 (37.5)	Parents both said to be normal with white father.
$\frac{mw}{m+}$	$\frac{mw}{M+}$	39	3511	3331	851 (902.1)	847 (795.9)	1584 (1581.9)	49 (51.1)	Parents' mother white.
$\frac{m+}{mw}$	$\frac{m+}{Mw}$	9	722	631	267 (272.8)	24 (18.2)	176 (180.6)	164 (159.4)	Parents' father white.
		132	11396	9905	4934		4969		

table were derived from earlier matings in which the normal parent was often heterozygous.

The sex ratio is very close to unity. The grand total of Table 1 is  $50.18 \pm 0.50$  % of males. For the first 50 families on the record  $\chi^2 = 51.02$ , expectations being calculated on a basis of equality. This is very close to the value of 50 expected if deviations from equality

<sup>1</sup> Including two gynandromorphs.

were wholly due to sampling. Where the ratio is aberrant, a sex-linked lethal might at first sight be postulated. Thus one family consisted of 41 ♀, 22 ♂, and it might be thought that a lethal gene had killed off about 19 potential males. But as 63 out of 67 eggs gave imagines, this is impossible.

Before discussing the segregation of white eye we must refer to the family whose maternal origin is prefixed by a query. This family segregated as if from  $\frac{mw}{mw} \text{♀} \times \frac{mw}{M+} \text{♂}$ . It is of course possible that a mistake was made as to the mother's eye colour. It is also possible that she was a mosaic, her eyes being dark, but her ovaries homozygous for *w*. All the other 109 segregating families segregated in approximately the expected ratios. Here the absence of normal males and the large number of white-eyed males are equally unexpected. This family will be referred to as the exceptional family.

The gene *w* for white eye is fully penetrant, and fully recessive on crossing. Since the sex ratio is unity we can legitimately add the data for the two sexes even when there was sex-linkage. The grand total for all back-crosses of known or presumed heterozygotes, including the exceptional family, is 2244 +, 2109 *w*, or  $48.45 \pm 0.76$  % white-eyed. The ratio is much the same when the total is subdivided into groups such as the progeny of white-eyed females and normal sons of white mothers.

The offspring from all crosses of known heterozygotes *inter se* is 2878 +, 1084 *w*, or  $27.36 \pm 0.71$  % white-eyed, a significant excess above 25 %. If the exceptional family is included, this becomes 27.91 %.

The single factor ratios are very steady from one family to another. Reckoning expectations of white-eyed mosquitoes as  $\frac{1}{2}$  and  $\frac{1}{4}$ , the 19 single raft cultures from heterozygous mothers gave  $\chi^2 = 11.23$ , the 26 from heterozygous fathers gave  $\chi^2 = 20.91$ ; and the 27 with both parents heterozygous gave  $\chi^2 = 19.63$ . This would be increased to 56.12 with *n* = 28, were the exceptional family included. With this exception, the variation between families is below that expected on a basis of sampling alone, though not significantly so.

*Linkage.* — Table 1 shows clearly that there is only one kind of heterozygous female, but two kinds of heterozygous male. The data agree with the hypothesis that females are *mm*, males *Mm*, and that *M* and *w* are closely linked, with a small recombination frequency *x*.

Thus the two types of heterozygous male may be symbolized as  $\frac{mw}{M+}$

TABLE 2.

Mother	Father	Expected types of segregation			
		$+ \text{♀}$	$w \text{♀}$	$+ \text{♂}$	$w \text{♂}$
$\frac{m+}{mw}$	$\frac{mw}{Mw}$	1	1	1	1
$\frac{mw}{mw}$	$\frac{mw}{M+}$	$x$	$1-x$	$1-x$	$x$
$\frac{mw}{mw}$	$\frac{m+}{Mw}$	$1-x$	$x$	$x$	$1-x$
$\frac{m+}{mw}$	$\frac{mw}{M+}$	$1+x$	$1-x$	$2-x$	$x$
$\frac{m+}{mw}$	$\frac{m+}{Mw}$	$2-x$	$x$	$1+x$	$1-x$

and  $\frac{m+}{Mw}$ , according as they derived the gene  $w$  from the mother or father. The expectations from the different types of segregating mating are given in Table 2.

The recombination frequency  $x$  is most simply calculated from the number of cross-overs among the progeny of heterozygous males of known parentage, mated to white-eyed females. These number 113 out of 2001, giving  $x = 0.056$ . However a somewhat better estimate can be obtained from all the families showing linkage. If we assume that the mother of the exceptional family bred as  $\frac{mw}{mw}$ , we have  $1-x : x : : 2862 : 185$ ; and  $1-x : 1+x : 2-x : x : : 1027 : 1011 : 1851 : 73$ . Applying the method of maximum likelihood, the logarithm of the likelihood is: —

$$L = 258 \log x + 1027 \log (1+x) + 3873 \log (1-x) + 1851 \log (2-x) + C.$$

Differentiating and putting  $\frac{dL}{dx} = 0$ , we have  $7009x^3 - 7470x^2 - 7801x + 516 = 0$ . So  $x = 0.0626$ . If the exceptional family is assigned to its putative mother we have  $7009x^3 - 7434x^2 - 7760x + 516 = 0$ , whence  $x = 0.0629$ . The expectations of Table 1 are calculated from the former value.

Were the uncertainty in the value of  $x$  wholly due to sampling we should have for its standard error,

$$\sigma^{-2} = \frac{-d^2L}{dx^2} = \frac{258}{x^2} + \frac{1027}{(1+x)^2} + \frac{3873}{(1-x)^2} + \frac{1851}{(2-x)^2} = 71648$$

whence  $\sigma = 0.0037$ . However most of the uncertainty is not due to sampling, as appears when we calculate  $\chi^2$ . Taking  $x = 0.0626$ , we have  $\chi^2 = 66.70$  for the 33 cultures in which a ratio of  $1-x:x$  is expected, and  $\chi^2 = 152.11$  for the 33 half-cultures of one sex in which a ratio of  $2-x:x$  is expected. The sampling distribution of  $\chi^2$  deviates from its classical form when expectations are small, but the probability that the above values should be due to sampling is extremely small. Most of the information about  $x$  is derived from back-cross families, and as  $\chi^2$  has twice its expected value, the amount of information is halved. The remaining information is even more reduced, so the standard error is about 0.006, or the recombination is  $6.26 \pm 0.6\%$ .

The cause of the divergences between different cultures is unknown. There were a few cultures with very high recombination. Thus two heterozygous brothers with white-eyed mates gave 16 cross-overs out of 99, and 12 out of 103. However brothers can differ greatly. Thus two brothers gave 11 out of 107 and 0 out of 96. The probability of obtaining so great a divergence by chance is  $\frac{192! 107!}{203! 96!} = 6.24 \times 10^{-}$

Other pairs of brothers show almost as large differences. The most obvious hypothesis is that there are inversions in the neighbourhood of the genes concerned which are occasionally lost by crossing over. This is however hard to reconcile with the data, and the question can only be decided by further genetical and cytological work.

*Gynandromorphs.* — Three gynandromorphs were found, A in a mass culture not included in Table 1, and B and C in separate cultures from  $\frac{mw}{m+} \text{♀} \times \frac{mw}{Mw} \text{♂}$ . A search of the literature revealed two other records (MARSHALL, 1938; WEYER, 1938) of gynandromorphism in this species, and 12 in all other mosquito species both in Britain and abroad. Of these, 7 were in the genus *Aedes* (EDWARDS, 1917; BRELJE, 1923; SHUTE, 1926; MARSHALL, 1938; SMYLY, 1942), 4 in the genus *Culex* (BEDFORD, 1914; MARSHALL, 1938; MIDDLEKAUFF, 1945), and 1 in *Theobaldia* (CLASSEY, 1942).

Our three gynandromorphs are described in Table 3. The mouth parts have not been included, as it has been found impossible to identify the stylets or their relative positions from the mounted heads. Fig. 1 shows the head of specimen B. Usually one appendage of a pair is

TABLE 3.

Organ	Left side	Right side
<i>Gynandromorph A.</i>		
Antenna	♂ normal	Torus as in normal ♀, flagellum hairier than normal ♀, less so than normal ♂.
Palp	♂ normal	Length intermediate between normal ♂ and ♀, 3rd, 4th, and 5th joints distorted, thickened, hairy.
Eye	Normal	Normal, slightly larger than left.
Fore leg, midleg	♂ normal	♀ normal
External genitalia	♂ normal	♀ normal
Gonads	Normal ovary	Normal ovary
Spermathecae	Two (normal ♀ has three)	
<i>Gynandromorph B.</i>		
Antenna	♀, hairier than normal.	♂ normal
Palp	♀ normal	♂ normal
Eye	Pigmented save for 15 white facets on median posterior margin.	White, no pigment.
Fore leg	♂ normal	♂ normal
Midleg	4th tarsal segment longer than 5th, claws toothed (as in ♂).	4th tarsal segment as long as 5th, claws toothed.
External genitalia	♀ normal	♂ normal
Gonads	Normal testis	Normal testis.
Spermathecae	Two	
<i>Gynandromorph C.</i>		
Antenna	♂ normal	♀ normal
Palp	Length between ♂ and ♀, distorted, thick, hairy.	♂ normal
Eye	Pigmented	Mainly pigmented, but groups of white facets give mottled appearance.
Wing	Smaller	Larger
Fore leg, midleg	♂ normal	♀ normal
External genitalia	♂ normal	♀ normal
Gonads	One ovary, one testis, but not certain on which side situated.	
Spermathecae	Two	

female, the other male, but it is not uncommon for one to be male and the other intermediate, as if a hormone diffused from the male regions. In *C* it will be noted that the right palp was male, the male appendage being on the left in other segments. The sex of the gonads has little relation to that of external organs.

Gynandromorphs *B* and *C* were members of otherwise normal cultures from  $\frac{m+}{mw} \text{♀} \times \frac{mw}{Mw} \text{♂}$ . Their interpretation is difficult because we do not know the sex of the eyes. Gynandromorphs in *Drosophila* generally arise by the loss of one *X* chromosome from some cells of

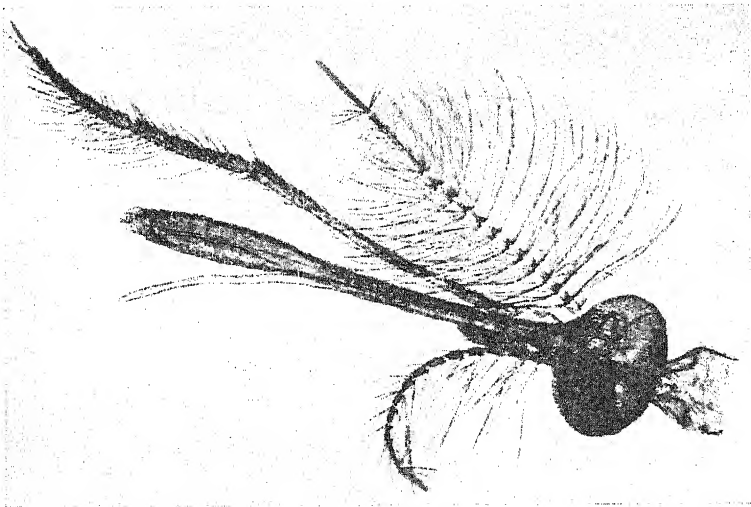


Fig. 1. Head of gynandromorph *B*. Explanation in Table 3.

what would otherwise be a female (*XX*). In a mosquito it seems quite likely that the loss of one of the six chromosomes would be fatal, and most unlikely that it would give normal female or male organs. We can at once rule out the possibility of dispermy alone. This would give a mosaic for sex but not for eye colour. On the other hand if a polar body were fertilized by a spermatozoön, and the female pronucleus by another, each providing nuclei for about half the body, the facts are explicable if one part is  $\frac{m+}{mw}$  and the other  $\frac{mw}{Mw}$ , or if one part is  $\frac{mw}{mw}$  and the other  $\frac{mw}{M+}$ . Such double fertilization seems to occur in *Lepidoptera* (GOLDSCHMIDT and KATSUKI, 1931).

If the gynandromorphs arose from a single diploid nucleus the

must have been  $\frac{m+}{Mw}$ . Elimination of a chromosome will not account for them unless we make the unlikely assumption that tissues with the gene  $M$  alone are female. But they can be explained on either of two hypotheses. Somatic crossing over may have occurred, as it does in *Drosophila*, giving rise to  $\frac{m+}{m+}$  and  $\frac{Mw}{Mw}$  regions. Or the two daughters of one chromosome may have gone to one pole at an early mitosis, those of its homologue going to the other. It is clear that such an event would be facilitated by somatic pairing. Either hypothesis implies that  $\frac{M}{M}$  is viable, as it is in *Ambystoma*, and that the white eye regions were male. This would have been in agreement with the appendages in *B* and with the palp in *C*. When genes appear which affect sexually dimorphic organs it will be possible to decide between these hypotheses.

*The influence of eye colour on behaviour.* — Normal imagines show a well-marked reaction when an opaque object such as a hand or a piece of paper is passed between the source of light and the cage. Mosquitoes which have settled on the netting of the cage at once become very active, making a loud buzzing when in flight. The reaction is of short duration but striking in intensity. In contrast, white-eyed imagines show no such activity.

When a bright light was switched off leaving a dimly lit room the normal imagines showed no such activity. The reaction is thus one to the moving contour between a bright and dark area of the visual field, and not to a decrease in light intensity.

However preliminary experiments by Mr. T. R. RAO in the Department of Entomology of the London School of Hygiene and Tropical Medicine have shown a response of both normal and white-eyed *C. molestus* to light intensity. Two mosquito cages, one covered with black paper, were placed about 12 inches apart in a room in daylight, and connected by a cardboard tunnel. About 100 mosquitoes, male and female, were put into the darkened cage, and the number of mosquitoes which had entered the lighted cage was counted at intervals. During the daytime both types showed little activity, and remained in the darkened cage. At dusk most of them came out into the uncovered cage. The difference in behaviour between the two types is exactly similar to that found by KALMUS (1943) between normal and white-eyed members of *Drosophila* species. Both types show phototaxis, but the white-eyed forms do not respond to moving visual contours, as their

ommatidia are not isolated from one another by pigment, and visual acuity is thus reduced to nil. However the light receptors are quite functional when it is irrelevant whether or not they are isolated.

In the larval and pupal stages eye pigment is wholly absent in  $\frac{w}{w}$  individuals; nevertheless no difference was found between their behaviour and that of normal individuals. Both showed a well marked shadow response when an opaque object was passed over the water; individuals suspended from the surface film immediately swam downwards. This reaction was also observed when, in a dimly lit room, a bright light placed near a bowl of larvae or pupae was switched off. It was therefore a response to light intensity, not to a moving contour.

*Discussion.* — The main result obtained is that in this species a pair of genes for which both sexes are diploid yet show a partial or incomplete linkage with sex. This phenomenon is not very rare. It is found in *Drosophila* species for the gene *bobbed*, which however at most crosses over very rarely with the sex genes (PHILIP, 1935). It occurs in the beetle *Phytodecta variabilis* (DE ZULUETA, 1925). Here the same sets of genes for natural polymorphism are found both in the X and Y chromosomes, and crossing over is very rare, if it occurs at all. In this species GALAN (1931) found an unequal pair of chromosomes in the male. So here, as in *Drosophila*, sex is determined by a chromosomal section, but the section of the X and Y chromosomes which is homologous contains homologous genes.

AIDA (1921) discovered partial sex-linkage in the Cyprinodont teleost *Aplocheilus latipes*, and it has since been found in other Cyprinodonts, notably *Lebistes reticulatus* where WINGE has studied it extensively. He found no unequal chromosomes in the male, nor did any of the numerous sex-linked genes which he found behave like those of *Drosophila* or mammals, where the Y chromosome does not carry them. On the contrary WINGE and DITLEVSEN (1938) were able to obtain viable »YY», or MM individuals provided that the Y chromosomes came from different lines. This suggests that the gene for maleness is often coupled with a recessive lethal, but not always with the same one. The fact that either sex may be heterogametic within a group of crossable species, or even within one species (WINGE, 1934), suggests that sex is here determined by genes, and not by chromosome segments.

The only species in which a number of genes showing both types of sex-linkage have been described is man (HALDANE, 1936, 1941).

Here the genes in that part of the *X* which has no homologue in the *Y* show the classical type of sex-linkage, while those which can cross over between the *X* and the homologous segment of the *Y* show partial sex-linkage, detectable in the progeny of heterozygous males as in *Culex*.

The strongest evidence for the determination of sex by a single gene is provided by the work of HUMPHREYS (1945) on the axolotl *Ambystoma mexicanum*. Here the male is normally *ff*, the female *Ff*. By grafting testes which were later removed, HUMPHREYS transformed *Ff* individuals into males. The mating *Ff* ♀ × *Ff* ♂ gave 3 ♀ : 1 ♂, and some of the females gave all female progeny with normal males, and were therefore *FF*. In this species femaleness therefore seems to be due to a single completely dominant gene. It is suggested that where sex is genetically determined in *Pisces*, *Amphibia*, many *Nematocera*, and probably *Reptilia*, it is usually determined by a single gene, even if we accept SVÄRDSON's (1945) presumptive evidence for a chromosome fragment determining sex in *Coregonus lavaretus*, which is not conclusive.

The fact that a worker so experienced as SUTTON in detecting small differences between homologous polytene chromosomes failed to find them in *Culex* suggests that if they exist they are at most of the slight nature associated with some single gene differences in *Drosophila*. An inversion which would allow several genes to act as a unit, or a deficiency or duplication of more than perhaps a single band would probably have been detected. The same applies to *Chironomus* and other Nematoceran species which have been extensively studied. A definite proof of the existence and viability of *MM* cells would strongly support the hypothesis of a single gene. In any case the sex-determining mechanism has as much right to be described as a gene as have such genes as *Bar*, *Delta*, or *Moiré* in *Drosophila melanogaster*.

On the assumption that sex is determined by a gene, or a small chromosomal abnormality which is not lethal when homozygous, and on the further hypothesis that somatic crossing over or abnormal mitosis occurs, we can perhaps explain the occurrence of spanogyny, or shortage of females, which ROUBAUD (1933) and TATE and VINCENT (1936) have observed in *Culex pipiens*. As a result of somatic crossing over mosquitoes or testes of genotype *MM* may arise, and their progeny will be entirely male. Spanandry could also be explained if other genes could determine sex, as they do in *Lebistes*. Lethal genes closely coupled with *m* could produce a shortage of females, but probably not families of 100 ♂ and 1 ♀ as reported by TATE and VINCENT.

Crossing over has so far only been detected in the spermatogenesis of *Culex*. It does not occur, except very rarely at mitosis, in *Drosophila* males. A comparison of its frequency in the sexes of *Culex* must await the discovery of two linked genes.

Although sex determination by a single gene is simpler than by a chromosome, and appears to be primitive in vertebrates, it cannot be assumed to be a primitive character in the *Nematocera*, if only because chromosomal sex determination is found in insect orders more primitive than the *Diptera*. Although the *Nematocera* are more primitive than the *Brachycera*, it is entirely possible that their ancestors had a sex determining mechanism like that of *Drosophila*. For STURTEVANT (1945) describes an autosomal recessive gene, *transformer*, in *Drosophila melanogaster* which has no effect on males, but transforms XX or even XXY zygotes which would otherwise be females, into males of normal morphology, apart from small size of the testes, and normal behaviour, but sterile. If a similar gene, or the same one with suitable modifiers, produced fertile males, it would be possible to secure a race in which the chromosomes of the sexes would be alike, and femaleness would be due to a single dominant gene.

The gene for white eyes in *Culex* is probably homologous with that in *Drosophila*, where there is only one locus giving this effect. If other sex-linked genes in *Drosophila* have sex-linked homologues in *Culex* it may be possible to homologize the whole or parts of the sex-determining chromosome of *Culex* and the X of *Drosophila*. If so this will be an argument for the primitive character of the condition in *Culex*, which could obviously evolve into the *Drosophila* mechanism by a simple process. So long as crossing over occurs between the chromosomes carrying *M* and *m*, or so long as *MM* testes occur with appreciable frequency, the chromosomal regions round the *M* locus are not shielded from natural selection. If, as the result of inversions, suppression of crossing over in the male, and suppression of somatic crossing over, these regions are shielded, recessive lethals, and ultimately deficiencies, will accumulate in them, and the *M* chromosome will degenerate into a nearly functionless Y. It is a striking fact that this has not happened in many large and successful groups.

The high variability of crossing over is the more striking since DE WINTON and HALDANE (1935) in *Primula sinensis* and SPURWAY (1945) in *Drosophila subobscura* found that crossing over was hardly more variable than was to be expected as a result of sampling, and decidedly less so than single factor segregation.

Our results emphasize the need for a widespread comparative study of sex-linkage. It is most unlikely that *Homo sapiens* is the only species in which both types of sex-linkage are found for a number of genes; and the partial type found in *Culex* may be far commoner than has been supposed.

We have to thank Professor P. A. BUXTON for allowing this work to proceed in his department, and for his interest in it; Dr. M. J. D. WHITE for photographing the head of a gynandromorph; and Dr. H. SPURWAY for suggesting the hypothesis here put forward as to the origin of the gynandromorphs.

*Summary.* — White eye in *Culex molestus* is a recessive character giving normal Mendelian ratios. It is partially linked with sex, showing  $6.3 \pm 0.6$  % recombination. There are no sex chromosomes, but maleness appears to be due to a single dominant gene in the same chromosome as that for white eye. Crossing over is unusually variable. Three gynandromorphs, two being mosaic for eye colour, are described. These latter cannot be due to chromosomal elimination, and may be due to somatic crossing over.

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# EXPERIMENTAL STUDIES ON THE INFLUENCE OF DDT INSECTICIDE UPON PLANT MITOSIS

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## I. INTRODUCTION.

THE c-mitotic activity of colchicine and some other substances has been one of the most central objects of cytological investigation during the last few years. At the same time as new efficient agents have been searched for, the physiology of the c-mitosis has been under careful examination. It may be considered as an undeniable merit of two Swedish investigators (LEVAN and ÖSTERGREN, 1943; ÖSTERGREN and LEVAN, 1943; ÖSTERGREN, 1944 a) that the many different parallel events related to the c-mitosis have been logically connected. The interpretation they give of the physiological mechanism of the c-mitosis will undoubtedly make an established basis for continued elucidation of this question.

The above-mentioned papers have established that in most organic substances the essential character of the c-mitotic activity is physical and therefore does not immediately depend on the chemical structure of a substance. According to LEVAN and ÖSTERGREN (1943) and ÖSTERGREN (1944 a), c-mitosis has many contacts with the narcosis phenomenon and may be considered as a kind of narcosis (in the widest sense of the word). Thus we are able to understand the c-mitotic activity of the most different organic substances in spite of their great variation in efficiency, i. e. the intensity of their visible effect. ÖSTERGREN (1944 a) distinguishes the following closely interrelated effects of organic chemicals on higher plants: *c-mitosis*, which can be *complete* or *partial*, *c-tumour formation*, *chromosome contraction* and many cases of *poison effect*.

It is, however, not necessary that all these related effects are visible at the same time. The active substance may thus be either efficient or inefficient as regards the individual effects. In certain cases other modes of activity can also be observed, though in the current literature there is little to be found on this aspect. These modes may probably

be specific to certain agents in some cases. As a whole, we are as yet very imperfectly acquainted with the above-mentioned phenomena.

When the present writer began to experiment with the DDT insecticide, which during the last few years had proved to be very effective, practical purposes were nearest in his mind. In practice it is often necessary to dust or spray DDT upon the surface of the plant buds at the critical stage of meiosis. Now, it might be thought that a possibly occurring c-mitotic or other effect could give rise to circumstances which would be noticed in practical farming. I have, however, undertaken to experiment with mitoses because, with them, the experiments are simpler to carry out, especially as it has been observed in the few cases studied that the same agents are active in meiosis also. Very probably these investigations will not contribute in any noticeable degree to the practical fight against the insect pests. However, the several interesting features observed, which are unfamiliar in the normal c-mitotic effect, ought to be worth a rather detailed description.

The DDT insecticide is a substance synthesized by the Swiss chemical factory GEIGY (Basle) and as a chemical compound is named dichloro-diphenyl trichloro-methylmethane. DDT is a contact insecticide that is very poorly soluble in water. According to LÄUGER, MARTIN and MÜLLER (1944), the influence of DDT upon the insects depends on its lipid solubility. Because of this property the substance is able to penetrate the epicuticula and chitin cuticula of the insects and enter into the ends of the nerves. There, it has a strong toxic effect. Its solubility in lipoids is considered to be based upon the chloroform radical, its toxic effect, on the other hand, upon the chlorobenzene part of the DDT molecule.

## II. MATERIAL AND METHODS.

As experimental objects I have used the common commercial varieties of the cultivated onion, *Allium Cepa* ( $2n=16$ ) and a leguminous plant *Trigonella foenum graecum* ( $2n=16$ ; cf. FRYER, 1930). The methods have been the same as those successfully used by the Swedish investigators (LEVAN and ÖSTERGREN, 1943) in their experiments with *Allium* and *Pisum*. The capacity of the glass jars in which the objects were growing was 30 cc. The DDT substance used (melting point  $106-107^{\circ}$  C.) was produced by Dr. T. VORTILA in the laboratory of the State Alcohol Monopoly, Helsinki, Finland. One of the series of the pure DDT substance has been made with the use of saturated tap-

water solutions. In order to bring about an easy dissolving the solutions have been made by pouring a small amount of saturated alcoholic solution of DDT into tap-water and boiling out the alcohol from the aqueous suspension.

Besides these solutions, others containing both ethyl alcohol and DDT have been made in such a way that a definite amount of DDT has been dissolved in a definite amount of absolute alcohol and the solution then poured into tap-water. The solubility of DDT is also quite low in ethyl alcohol. The solutions contain, therefore, rather large amounts of alcohol, i. e. 1, 2, 3 and 4 cc. per 30 cc. water, corresponding to 3,3, 6,7, 10 and 13,3 vol. %. In order to investigate the influence of the pure ethyl alcohol also an alcohol series of corresponding concentration was used.

The root-tips were fixed with twenty-four hours' intervals after 1, 2 and 3 days; in the pure DDT solutions also after 4, 5 and 7 days. The only fixative used was the craft modification according to RANDOLPH.

Exclusively the paraffin method has been used during the investigation and mainly longitudinal sections were made. Staining was partly done by the common iodine-gentian-violet method. As in the course of the examinations it appeared necessary to distinguish between the nucleolus and the chromosomes, recourse was also had to a differential staining. At first I attempted to use the differential staining method of SEMMENS and BHADURI (1939), in which the chromatin is stained by the FEULGEN method and the nucleoli with light-green. However, as the light-green-stain was found to be extracted too easily during the differentiation procedure, I tried to combine the common iodine-gentian-violet staining with the FEULGEN method, and the results have been satisfactory.

After being removed from the fuchsine-sulphurous acid and passed through the sulphurous acid water the slides were immediately stained according to the common iodine-gentian-violet method. For the purpose of obtaining the desired tint, the gentian-violet stain must be rather weak in order not to conceal the FEULGEN stain. Therefore, it is best to stain only a short time (2 min. for *Allium* and 5 min. for the less stainable *Trigonella*) and to continue the differentiation in absolute alcohol so long that the FEULGEN stain beneath becomes dimly visible at a macroscopical inspection. The most reliable result can be attained, however, by controlling the differentiation process under the microscope. The chromatin comes out as dark purple, the nucleoli as bluish green. This staining method, however, does not admit of general application, and

the desired result can only be reached by using root-tips treated with ethyl alcohol and DDT + ethyl alcohol. On untreated slides or slides treated only with pure DDT the nucleolus either fails to stain or the stain disappears during the differentiation.

A different gentian-violet staining of euchromatin and heterochromatin at various mitotic stages could clearly be observed. In the euchromatic parts of the resting nuclei the pure red colour of the FEULGEN stain could be seen, while the heterochromites were a very distinct purple. In the cases where the matrix substance was diminished at metaphase the heterochromatic parts of the chromosomes could also be distinguished at these stages.

### III. THE RESULTS.

#### 1. ALLIUM CEPA.

*The pure DDT substance.* — As above mentioned, saturated tap-water solutions were used in all experiments. The first fixation was made after twenty-four hours' treatment. After this time of treatment, as well as after all other times up to seven days, no c-mitotic effect except the extra chromosome contraction could be observed. This is on an average 30 % of the total length of the chromosomes. In other respects the divisions were normal.

The toxic effect of the DDT substance seems to be weak. The roots were still fully turgescient after treatment lasting seven days. After two weeks, however, the roots seem to be killed.

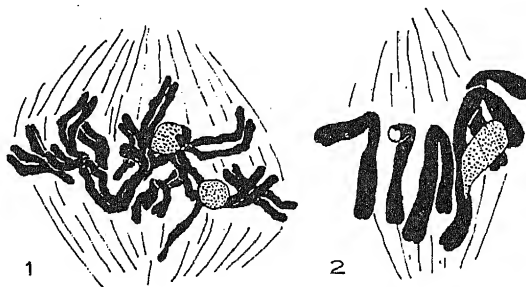
The pure DDT substance is, thus, a weak mitosis-disturbing agent, the effect of which reaches its maximum in producing the extra chromosome contraction. According to ÖSTERGREN (1944 a, p. 443), this effect is the only visible expression of the c-mitotic properties, which we can see when using the lowest concentrations giving effects in the experiments. The cause of the absence of other c-mitotic effects in the present case — such as are usually brought about by stronger concentrations — is the poor solubility of the DDT substance. This makes it impossible to use sufficiently strong concentrations. In this respect DDT substance can be compared with such substances as diphenyl, azobenzene, dimethylaminobenzene, MICHLER's ketone, phenanthrene and anthracene, with which experiments have been conducted by ÖSTERGREN (l. c.).

The toxicity threshold of the DDT substance also lies obviously

above the water solubility limit in the saturated solutions. In this respect the substance is reminiscent of the likewise very poorly soluble acenaphthene (ÖSTERGREN, l. c.).

*Ethyl alcohol.* — As earlier mentioned, in the pure ethyl alcohol series the concentrations of 3,3—13,3 vol. % were used. In the lowest concentrations the true c-mitotic disturbances affecting spindle function were rarer than in higher ones, nor was the intensity of the other disturbances comparable with that in the strongest solutions. ÖSTERGREN (1944 a, p. 446) mentions the c-mitotic threshold concentration of ethyl alcohol as 1,0—0,5 mol./l, corresponding to the concentration of about 5,8—2,9 vol. %. Thus the results obtained correspond closely with the values published by him. Neither can any macroscopically visible

Figs. 1—2. *Allium Cepa*. — Fig. 1. A metaphase side view of mitosis treated with ethyl alcohol. Two nucleolar bodies. The visible structure of the chromosomes is not drawn. — Fig. 2. A part of the metaphasic chromosome set of an untreated plant. The nucleolus attached to a chromosome is visible. —  $\times 1850$ .



poison effect be observed during the first few days in the highest concentrations.

In the alcohol series an extra chromosome contraction corresponding to that found when the pure DDT substance is used can be observed. The average contraction is also the same, i. e. 30 % of the normal chromosome length.

Besides the chromosome contraction, some features peculiar to the partial c-mitosis have also been observed. As characteristic of these partial c-mitoses the metaphase chromosomes are arranged in the middle of the cell. In several cases a rather complete metaphase orientation may occur, even when the higher alcohol concentrations are used. No achromatic sphere on the surface of which the chromosomes are placed (SHIMAMURA, 1939; BERGER and WITKUS, 1943) can be observed.

No X-shaped metaphase chromosomes characteristic of the metaphase stage of the full c-mitosis can be seen in these partial c-mitoses. The chromosomes are clearly divided into two chromatids lying parallel

to one another during metaphase (Fig. 1). The failure of the centromeres to divide at metaphase of the full c-mitosis has an influence on the elastic repulsion (ÖSTERGREN, 1943) between the two chromatids. Those forces which produce X-shaped chromosomes have not been at work in this case.

Characteristic of the partial c-mitosis is (ÖSTERGREN, 1944 a) the fact that the spindle apparatus is not so much disturbed as to prevent anaphase, but the spindle is unable to give a bipolar anaphase. This is the common case in *Allium* treated with ethyl alcohol. The chromatids separate, sister-nuclei are formed and a cell-wall arises between nuclei. No formation of polyploid cells peculiar to the full c-mitosis can be observed.

In the root-tips treated with alcohol very important changes are observed in the matrix or kalymma surrounding the chromonema, i. e. in the substance containing nucleic acid and basic proteins around the chromonema (CASPERSSON, 1936, and later with collaborators; SERRA, 1942, 1944). Already at prophase, when the accumulation of matrix substance begins in the normal chromosome cycle, we can see that the formation of matrix is weaker than usual. The chromatids are seen to be already separate at an early stage. The spiral structure of the chromonema is quite clearly visible. Lack of matrix substance continues during the whole mitosis. At telophase there then follows the definitive disappearance of matrix belonging to the normal chromosome cycle. The literature seems to contain but few reports of corresponding observations. It is, however, very probable that the same phenomenon can be brought about by other agents too. LEVAN (1945) has observed a corresponding influence from several inorganic salts. BERGER and WITKUS (1943, Plate 1) have published some micro-photographs of c-mitoses induced by colchicine in *Allium*. These show in my opinion a lack of matrix substance similar to that mentioned above. In their experiments with liquid benzene and benzene vapour BERGER (1944) and BERGER, WITKUS and SULLIVAN (1944) have particularly mentioned the phenomenon under consideration.

It can be assumed that the matrix substance of the chromosomes has become more fluid owing to the treatment with ethyl alcohol. This phenomenon is named by RESENDE (1941) »chromatic agglutination». The viscosity of the substance has decreased and it follows that the chromosomes and particularly the distal ends of them have acquired a tendency to glue together, causing the sticky phenomenon. This manifests itself at the anaphase separation of the chromatids when

»false» bridges arise between the two chromatid-sets. In the root-tips treated with alcohol the sticky phenomenon is, however, less pronounced than in those treated with DDT + alcohol, as will be shown later. By no means all anaphases show the sticky phenomenon, and the false bridges are no longer visible after anaphase.

An important feature is also the occurrence of nucleoli even after prophase. This phenomenon, like the stickiness of the chromosomes, is also more intensive after treatment with DDT + alcohol.

The nucleolus behaves during prophase entirely normally, i. e. it diminishes in bulk at the end-stage of prophase so that by the same time the nuclear membrane — or nuclear-cytoplasmic surface, as it has been named by DARLINGTON (1937) — disappears the nucleolus has become very small or has wholly disappeared. Irrespective of that, most of the metaphase figures show nucleoli fastened upon the chromosomes (Fig. 1). The number of nucleoli is usually one or two, more rarely three or four. In the alcohol series the nucleoli disappear wholly at anaphase or at latest after the beginning of telophase, probably being resorbed into the cytoplasm. At telophase no lagging nucleoli between the nuclei could be seen. The formation of the new nucleoli beginning at telophase occurs entirely normally, i. e. the nucleoli begin to appear as a rule at two points inside the group of the telophasic chromosomes. The so formed nucleoli as a rule fuse very soon to one large body.

The fibre structure of the spindle does not in any way deviate from the normal.

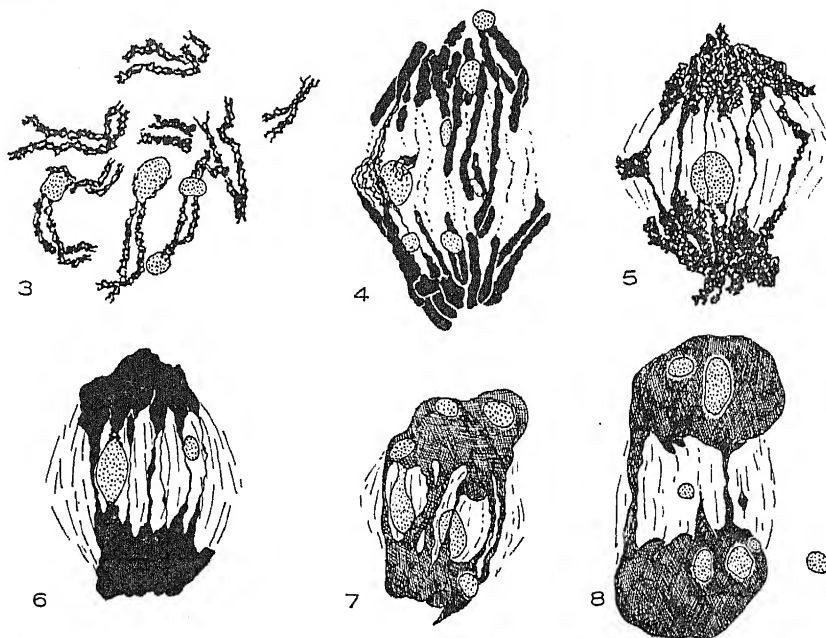
*The DDT substance + ethyl alcohol.* — In the solution series prepared of DDT suspensions of different concentrations the variation in the amount of the suspended substance has no influence. Neither do the different treatment-times — the shortest time was twenty-four hours — cause any essential differences in the quantity or quality of the effect. On the other hand, in the solutions of the lowest alcohol concentration (3,3 vol.%) the c-mitotic effects were weaker and the regular metaphase orientation occurred quite often.

The extra chromosome contraction has always been observable. In amount it corresponded closely to that mentioned above in connection with the pure DDT and the pure ethyl-alcohol treatment. This contraction was on an average 22 % of the normal length of the chromosomes.

At metaphase we can observe the same c-mitotic effect as after alcohol treatment, though more distinctly expressed. The regular metaphase orientation cannot be observed in any case except that of the

lowest alcohol concentration. The chromosomes are always scattered in the middle of the cell (Fig. 3). This figure also gives us a conception of the shape of the chromosomes at this stage. X-shaped chromosomes are entirely lacking. The chromatids lie parallel and they very often seem to be quite separate. They are frequently relationally coiled.

The spindle apparatus is often functional, as can be seen from the typical anaphase and the normal separation of the chromatids. In



Figs. 3—8. *Allium Cepa*. DDT + ethyl alcohol treatment. — Fig. 3. A part of a metaphase plate with scattered chromosomes. Four metaphase nucleoli, lack of the matrix, and the two-partite structure of the chromatids are visible. — Fig. 4. A false bridge and many parts of the metaphase nucleoli fusing with chromatids are visible. A chromatin elimination attached to the largest nucleolus is to be seen. The structure of the chromatids is not drawn. — Fig. 5. A late anaphase with false bridges. — Figs. 6—8. Telophases with numerous false bridges. In Fig. 8 a small chromatin elimination and one metaphase nucleolus pushed out from the division figure are visible. —  $\times 1850$ .

several cases, however, the occurrence of multipolar spindles can be observed, which feature according to ÖSTERGREN (1944 a) is very characteristic of the partial c-mitosis. Formation of polyploid cells has to some extent occurred. The disturbances have thus in several cases led to full c-mitosis.

The weak development of matrix substance like that mentioned

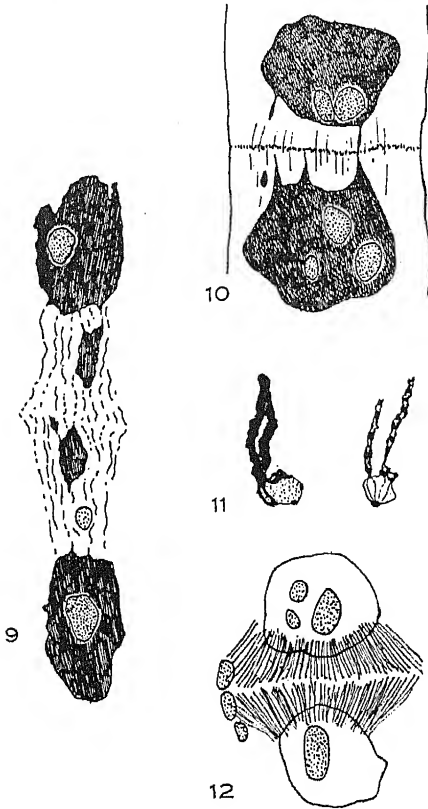
above in connection with the pure alcohol treatment is even more pronounced. At met-anaphase the chromatids seem to be quite »naked» as at the resting stage (Fig. 3) and the chromonemic structure of the chromatids is clearly visible. The two-parted structure of the chromatids is to be indubitably seen (cf. LEVAN, 1945). Whether the half chromatids are further divided, and what the structure of the chromonema is (cf. NEBEL, 1939), has not been examined in this connection.

The »chromatic agglutination» and the sticky phenomenon originating from it are regularly visible after DDT + alcohol treatment both at ana- and telo-phase. The intensity of the stickiness compared with alcohol treatment is also increased. Figs. 4—10 give us a conception of the phenomenon from anaphase to the end stage of telophase. It can be observed that the gluing of the chromatids occurs principally end to end, i. e. the distal ends of the chromatids have the strongest tendency to fuse. In some cases (Fig. 5, the outermost bridge to the left) we can, nevertheless, see that the larger pieces of the chromatids also are able to fuse. The anaphase separation stretches the fused chromatids so that numerous false bridges arise. The durability of the bridges in the DDT + alcohol series is much greater than those after alcohol treatment. They usually last until late telophase (Fig. 8). These bridges rarely occur during the formation of the cell-wall between the sister-nuclei (Fig. 10). This feature seems to be typical of the bridges induced by ÖSTERGREN (1944 b) with ethylene glycol.

The bridges break between the delayed chromosomes and give to the sister-nuclei a very irregular shape. It is impossible to say whether the bridges may sometimes separate intact. Some features, however, suggest that at the break the one suffers deletion while the other gets a duplication. It happens very often that stretching connected with the stickiness breaks the chromatids in such a way that some of their pieces are eliminated. The eliminations visible in Fig. 9 are uncommonly large, in general they are smaller, as can be seen in Figs. 8 and 10. The rôle of the lagging nucleoli in these eliminations must also be mentioned.

The nucleoli appearing after prophase are essentially similar to those found after the alcohol treatment, and their frequency is increased in this case too. In the nucleolar cycle from telophase via resting stage to prophase no deviations from the normal can be observed. Here, too, at the end-stages of prophase the nucleoli are either very insignificant or have wholly disappeared. At the metaphases, however, we can regularly see nucleoli attached to the chromosomes

(Fig. 3). Their number is in general 2—4. In view of the fact that the prophase nucleoli are resorbed in the normal way before the disappearance of the nuclear-cytoplasmic surface *we must consider the metaphasic nucleoli as new formations and not as residues of the foregoing nucleolar cycle*. I have not found in the literature any corresponding case, but RESENDE (1938, p. 393) draws attention to its prob-



Figs. 9—12. *Allium Cepa*. DDT + ethyl alcohol treatment. — Fig. 9. A late telophase with exceptionally large chromatin eliminations. — Fig. 10. The false bridges have persisted until the formation of the cell-wall. Observe the asymmetry of the true nucleoli in the sister-nuclei. — Fig. 11. The formation of the metaphase nucleoli in the chromosomes. In the chromosome to the left the visible chromonema is not drawn. To the right, two fibrils are discernible corresponding to the two chromatids. — Fig. 12. Some lagging nucleoli and the coarsened structure of the spindle are visible. —  $\times 1850$ .

ability in one case observed by him. The bulk of the nucleoli increases during metaphase and is often rather considerable at anaphase (Figs. 6 and 7). During metaphase we can frequently see that many of the chromosomes are glued to the nucleoli. It may also occur that two nucleoli at metaphase fuse to one larger body. They thus connect the chromosomes producing the nucleoli with other chromosomes from the vicinity.

In contradiction to the conditions after alcohol treatment the nu-

cleoli are not resorbed at anaphase but remain and, in addition to the stickiness, cause a more difficult separation of the chromatids (Figs. 6 and 7). Very frequently during the separation of chromatids the nucleoli break into many pieces and these fasten at haphazard on the chromosomes (Fig. 4). At the end of anaphase, however, the nucleoli are released from the chromosomes and small parts of the anaphase chromosomes follow them in many cases (Fig. 4, the large nucleolus to the left). The loosened nucleoli are resorbed very slowly, lagging at telophase (Figs. 8, 9 and 12). Some of the lagging nucleoli can, however, become enclosed in the arising sister-nuclei (Fig. 8, the smallest nucleolus in the nucleus below). However, that these nucleoli should continue their existence and take part in the forming of the normally functioning nucleoli is improbable. At telophase the nucleolar cycle begins anew, the nucleoli being formed on particular regions of the chromosomes. The symmetry between the nucleoli that have arisen in the sister-nuclei, as observed by HERTZ (1931 a and b) and others at the normal mitoses, has been seriously disturbed (Figs. 8, 10 and 12). This is, however, quite intelligible, as the telophasic polarity is disturbed owing to the abnormal mitosis.

It is difficult to see on which chromosomes and in what manner the metaphase nucleoli arise, as they are connected with other chromosomes as well as those forming them. Still, in favourable cases we can find some elucidating details.

In the normal mitoses of *Allium* we usually find two nucleoli which arise at the SAT-constrictions of the satellited chromosome pair (cf. HERTZ, 1932, p. 597). We can frequently see, however, three nucleoli at the same time. It may thus be assumed that, besides the ordinary SAT-constrictions, also other constrictions take part in normal circumstances in the forming of nucleoli.

It is impossible to identify the nucleolar chromosomes at metaphase, especially as the disappearing of the matrix substance destroys the normal shape of the chromosomes. The number of the metaphase nucleoli (from one to five) indicates that the satellited chromosomes cannot be the only organizers of the nucleoli. On the contrary, it is very possible that the organizers of true nucleoli, the SAT-constrictions, do not at all take part in the forming of the metaphase nucleoli.

In most cases the metaphase nucleoli arise on the distal ends of the chromosomes (Figs. 1, 3 and 11), but occasionally also the intercalary location seems to be possible (Fig. 3, the outermost nucleolus to the right). One may often get the impression that the nucleoli arise as

terminal knobs on the ends of the chromosomes, as DOUTRELIGNE (1933) has stated. The favourable figures show, however, that this is not the case. The nucleolus has arisen subterminally on a zone intercalary to a part of the chromosome much resembling a satellite (Fig. 11). Obviously, this small piece is frequently eliminated with the lagging nucleoli at ana-telophase. The nucleolar substance swells out of its proper place of origin, occasionally even filling long gaps between the chromatids.

In some cases the nucleoli contain very thin fibrils that give the FEULGEN reaction. The chromosome to the right in Fig. 11 represents a case where these are very clearly visible. It could be observed that each chromatid corresponds to two fibrils inside the nucleolar body. The existence of the fibril-like structures in the nucleolar regions of the chromosomes, i. e. the continuation of the chromonema in the heterochromatic region also, has been observed in many cases (cf., e. g., KAUFMANN, 1938). The number of the chromonemata — two per chromatid — corresponds with the observations of GATES and PATHAK (1938) in *Crocus*.

As HEITZ (1932) has already stated, the distal ends of the somatic chromosomes of *Allium* are heterochromatic. In this investigation the same condition has been observed, which seems to occur frequently in the chromosomes of several organisms (cf., e. g., KOSTOFF, 1938 a and b), DARLINGTON (1937) has also pointed out that the distal ends of the chromosomes have a tendency to become heterochromatic and inert, i. e. the telomeres assume a deviating character from the other parts of the chromosomes. *The origin of the metaphase nucleoli may thus be connected with the heterochromatic telomere parts of the chromosomes.*

After DDT + alcohol treatment certain changes in the spindle structure have been quite frequently observed. In the slides stained with iodine-gentian-violet a very distinctly stained spindle can be seen (Fig. 12). With FEULGEN staining the structure becomes invisible.

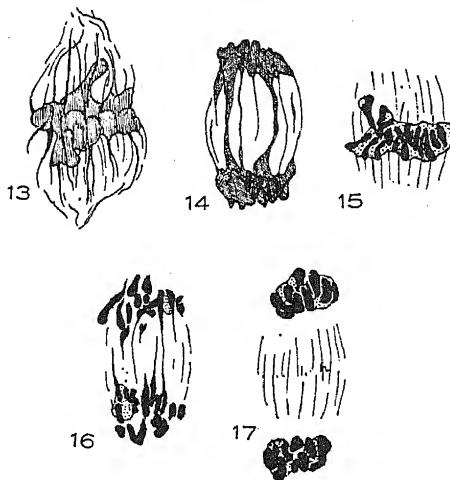
## 2. TRIGONELLA FOENUM GRAECUM.

*Ethyl alcohol.* — Here we have the same alcohol concentrations as with *Allium*. The investigated mitoses are rather normal. It has been impossible to measure the length of the chromosomes, and hence any c-mitotic chromosome contraction that may occur cannot be observed. The formation of metaphase plates is regular. The metaphase chromosomes are to a certain extent more swollen than the normal ones, indicating the presence of nucleolar substance on their surface. Any

stickiness, however, does not occur at anaphase, which is regular. The spindle structure is a little more distinct than normally.

*The DDT substance + ethyl alcohol.* — The same series as with *Allium* have been used excluding, however, the lowest alcohol concentrations, 3,3 and 6,7 vol. %. No true c-mitotic effects can be observed, the chromosome orientation being quite regular. Especially at metaphase in the slides stained with iodine-gentian-violet the chromosomes are, however, swollen and glued together in such a way that the individual chromosomes cannot be distinguished (Fig. 13). The corresponding effect has been observed by ÖSTERGREN (1944 b) in *Allium*

Figs. 13—17. *Trigonella foenum graecum*. DDT + ethyl alcohol treatment. — Fig. 13. A metaphase side view and the coarsened spindle structure. Nucleolar substance covers the whole chromosome set. — Fig. 14. An anaphase with false bridges. Nucleolar substance surrounds the chromosomes. — Fig. 15. A metaphase side view. Nucleolar substance and chromosomes are separately visible. — Fig. 16. An anaphase. The stickiness of the chromosomes and two rather large metaphase nucleoli are to be seen. — Fig. 17. A late telophase. Metaphase nucleoli have disappeared. Two true nucleoli have arisen. — Figs. 13 and 14 are stained with iodine-gentian-violet, Figs. 15—17 with FEULGEN + gentian-violet differential stain. —  $\times 1850$ .



treated with ethylene glycol. During anaphase the same phenomenon continues, though it is not so conspicuous as at metaphase. Further, at anaphase we can very frequently see stickiness with many false bridges (Fig. 14). The metaphase nucleoli seem, however, to be lacking.

Only the differential staining FEULGEN and gentian-violet revealed that the same conditions as were found in *Allium* are also present in *Trigonella*. It appeared that the swelling and agglutination of the metaphase chromosomes are caused by a nucleolar substance which covers the metaphase chromosomes (Fig. 15). The picture is reminiscent of the cases mentioned by MCCLINTOCK (1934) at the meiosis in *Zea* where the nucleoli arise without nucleolar organizers. As the nucleolar cycle of the ordinary nucleoli of the treated *Trigonella* normally begins

from telophase via the resting stage to the end of prophase, we must regard the metaphasic aggregation of nucleolar substance as corresponding to the metaphase nucleoli in *Allium*.

At the anaphase of *Trigonella* no lagging nucleoli as in *Allium* can be observed. On the contrary, during the anaphase separation the nucleolar substance remains fixed upon the surface of the chromosomes (Fig. 14), disappearing at the same time rapidly. In Fig. 16 we see an anaphase where, of the nucleolar substance, there remain two larger bodies in addition to thin layers on the chromosomes which have been impossible to reproduce on the drawing. During telophase as a rule the nucleolar substance is wholly resorbed. An attraction between the chromatids and nucleolar substance seems to be so much greater in *Trigonella* than in *Allium* that separate rounded nucleolar bodies cannot arise.

At telophase the normal nucleolar cycle begins anew. The nucleolar substance has arisen at two points (Fig. 17), which probably correspond to the satellite chromosome pair of *Trigonella*. In general the two nucleoli fuse very soon together to one larger mass.

For examination of the amount of the matrix substance *Trigonella* is not a suitable object owing to the small size of the chromosomes. Using the FEULGEN stain it has, however, in many cases been possible to observe the chromomeric structure of the chromosomes after DDT + + alcohol treatment. Without treatment this was impossible. This indicates clearly the corresponding disappearance of matrix substance as in *Allium*.

In the *Trigonella* material stained with gentian-violet the thickening of the spindle fibres is somewhat more common and distinct than in *Allium*. The metaphase represented in Fig. 13 reveals the spindle structure very clearly.

### 3. SUMMARY OF THE OBSERVATIONS.

Table 1 gives a summarized account of the frequency and intensity of the different effects both in *Allium* and in *Trigonella*. The frequency has been represented by means of asterisks, three asterisks signifying *regularly*, two asterisks *frequently* and one asterisk *rather rarely*. The different degrees of the intensity are marked with three, two or one cross. In the alcohol and the DDT + alcohol series only the highest alcohol concentrations, i. e. 10 and 13.3 vol.%, have been compared.

The table shows that treatment with pure ethyl alcohol has almost exactly the same effects as that with DDT and ethyl alcohol together.

TABLE 1. *The different effects of ethyl alcohol and DDT upon the mitosis.*

Effect	<i>Allium Cepa</i>			<i>Trigonella foenum gracum</i>	
	Pure DDT	Ethyl alcohol	DDT + ethyl alcohol	Ethyl alcohol	DDT + ethyl alcohol
Chromosome contraction	++	++	++	?	?
C-mitosis .....	—	+	++	—	—
Disappearing of the matrix substance .....	—	++	+++	—(?)	++
Sticky phenomenon at ana- telophase .....	—	**	***	—	***
Occurrence of metaphase nucleoli .....	—	**	***	***	***
Occurrence of nucleolarbo- dies at anaphase .....	—	++	+++	+	+++
Lagging nucleolar bodies at telophase .....	—	*	***	*	**
Thickening of the spindle structure.....	—	—	***	—	—
	—	—	**	—	***

The DDT substance together with ethyl alcohol causes, however, a very clear increase in the frequency and intensity of these effects.

Both in *Allium* and in *Trigonella* a significant difference between the nucleolar substance at metaphase and the normal true nucleoli can be observed. The true nucleoli are always clearly vacuolized. In the metaphasic nucleolar bodies, on the contrary, vacuoles have never been observed but these bodies remain entirely homogeneous.

#### 4. THE SUBSEQUENT EFFECTS OF THE DDT + ETHYL ALCOHOL TREATMENT.

In order to examine the subsequent effects of the treatment with DDT + ethyl alcohol some onions were placed in pure tap-water after a three-days' treatment. Fixations were made after one and two days.

Slides from the two fixations show the metaphasic disturbances to continue similarly to those found during the treatment. The true c-mitotic disturbances have diminished, i. e. the formation of metaphase figures is quite regular. Metaphase nucleoli, however, arise as intensively as earlier. There is now less stickiness and the amount of matrix sub-

stance is more normal. In the new root-tips developed in water the divisions occur normally.

#### IV. DISCUSSION.

Of the effects produced by DDT, ethyl alcohol and, above all, these two substances in combination, we would draw attention especially to three, viz. the lack of matrix substance or kalymma, the stickiness of the chromosomes and the arising of nucleolar substance at metaphase. All these phenomena are known to the cytological literature but, as simultaneous phenomena, they have not been observed earlier. It is, therefore, interesting to examine whether these different effects, which the investigations of the last few years have elucidated more effectively, have any causal connection with one another.

It is clear that a very significant cause of the abnormalities mentioned is the specific effect of the chemical compounds used. We know even a great group of c-mitotic active substances that are not able to effect the phenomena in question. Some preliminary experiments made with *Kniphofia aloides* have, however, demonstrated that the same concentrations of ethyl alcohol and DDT as used in *Allium* and *Trigonella* have no effect. At the same time the c-mitotic disturbances are very weak. According to these observations, we have reason to suppose that there also exist other factors which co-operate with the specific chemical effect.

References to the occurrence of nucleoli at the mitotic division stages after prophase are rather common in the literature. In this connection mention may be made of VAN CAMP (1924), YAMAHARA and SINOTO (1925), ZIRKLE (1928), DERMEN (1933), SATO (1936), RESENDE (1937), RAMANUJAM (1938) and HÅKANSSON and LEVAN (1942). Corresponding meiotic nucleoli have been observed by, e. g., DERMEN (1933) and FRANKEL (1937). The occurrence of the nucleoli has, however, been described as a more or less sporadic phenomenon. In some cases, as for instance according to LEVAN (HÅKANSSON and LEVAN, l. c., p. 439) in Leguminosae plants, it can be very common. RAMANUJAM (l. c.) has noticed the phenomenon quite regularly at mitoses of *Oryza*. Some of these observers state that the nucleoli persist from the foregoing prophase. RESENDE (1938) only reports the possibility that in the case published by him (1937) it might be a question of a new nucleolus. The regularity and the precocity in the occurrence of the metaphase nucleoli were thus previously unknown phenomena.

On account of the classical investigations of HEITZ (1931 a and b)

it has become clear that the normal nucleoli are formed by definite parts of the chromosomes, i. e. the SAT-constrictions. This fact has been verified so often that in spite of some contradictory observations (e. g., DERMEN, 1933; DOUTRELIGNE, 1933) it cannot be questioned. The investigations of MCCLINTOCK (1934) have to a great extent elucidated the function of these nucleolar regions. Above all it has been made clear that the activity of the nucleolar regions is genically controlled. RESENDE (1938) has criticized the interpretations of MCCLINTOCK, and he ignores the nucleolar-substance-producing function of the nucleolar regions. He thinks these to be nucleolar-substance-collecting organs in which the true nucleolus arises from material called by him pre-nucleolar substance. The occurrence of this depends on the existence of heterochromatin in the cell.

HEITZ (1931 a), by establishing the lack of nucleic acid in the nucleolar region and naming them SAT- (Sine Acido Thymonucleinico-) constrictions, has really verified the heterochromacity of them. Since then the relation between heterochromatin and nucleoli has been still more clearly expressed (cf. SERRA, 1942; HUSKINS, 1942; STRAUB, 1943). Cytology has at last escaped the obscurity and the speculations (cf. DERMEN's review, 1933, on the subject) which have dominated the question of the function of nucleolus. Especially the investigations of CASPERSSON (1940, and later with collaborators) have made it possible to establish a hypothesis on the probable mode of function of the nucleolus.

DARLINGTON and LA COUR's (1938, 1940) observations on the so-called »differential segments» have shed new light on the problem of heterochromatin. According to them, these are heterochromatic regions which do not become visible at normal metaphases but after cold treatment can be seen as unstained zones in several parts of the chromosomes. This so-called negative heterochromacy (KLINGSTEDT, 1941) is caused by a certain »starvation» of nucleic acid of the heterochromatin. The other investigators have also observed a similar »allocyclus» of heterochromatin in plants (GEITLER, 1940 a; TSCHERMAK, 1943) as well as in animals (KLINGSTEDT, 1941; WICKBOM, 1945). The proximate cause of allocyclus in the case represented by KLINGSTEDT was the hybridity of the animals.

It is, however, as yet unexplained what the relation is, on one hand, between the above mentioned differential segments and the nucleolar regions and, on the other hand, between the last-mentioned and the non-SAT-constrictions (RESENDE, 1938) which take no part in

the formation of the nucleoli. LEVAN (1942) thinks that secondary constrictions in the haploid rye observed by him correspond to the differential segments. KLINGSTEDT (1941) has in an interesting way dealt with this question. As a difference between euchromatin and heterochromatin he mentions the timing difference shown by the nucleic-acid cycle of the two substances. The nucleic acid of euchromatin disappears after telophase, becoming again distinctly visible at next prophase. In this way the nucleic-acid cycle of heterochromatin is behind — or perhaps rather before — the cycle of euchromatin. The disappearance of the nucleic acid occurs so near the beginning of the new synthesis of nucleic acid that the achromatic phase practically does not exist. External agents, e. g. low temperature, can cause a delay in the synthesis of new nucleic acid and thus affect the visibility of the differential segments at this period. KLINGSTEDT (l. c., p. 173) mentions that great similarity to the nucleolar regions, and says: »One may perhaps tentatively suggest that the nucleolus organizer is a special kind of heterochromite, the productive activity of which is postponed until telophase and consists in a condensation of chromosome products so much changed as not to show the staining reactions of thymonucleic acid any more».

DARLINGTON (1941) has observed, in some *Paris* species, the occurrence of differential segments to be genically controlled. In some cases such conditions exist that allocyclus cannot appear simultaneously with the nucleolar regions. He mentions, it is true, also the existence of both phenomena at the same time. GEITLER (1940 a), TSCHERMAK (1943) and WICKBOM (1945) have also observed the same case in several organisms.

Though it is clear that heterochromatin forms a very heterogeneous group of substances, it seems obvious that *we can find several functional similarities between nucleolar regions (= SAT-constrictions), non-SAT-constrictions and differential segments*. They are all heterochromites, the nucleic-acid cycle of each being specially differentiated. The capability to form nucleoli may also be similar. If we want to widen the above-mentioned hypothesis of KLINGSTEDT we must draw out the conclusion that the nucleolar regions and non-SAT-constrictions are heterochromites having an allocyclus that is permanently changed. The nucleolar regions are differentiated as places where the true nucleoli as a rule arise. The non-SAT-constrictions may also be able to form nucleoli. That this may often occur can be seen from the fact that the number of nucleoli is greater than that of SAT-constrictions (cf.

DERMEN, 1933). Differential segments, on the contrary, cannot as a rule be distinguished owing to failure of the necessary intervening time in their nucleic-acid cycle. Their heterochromatin is, however, as usual (cf. SERRA, 1942) very sensitive to changes of the external and internal factors. In this case the sensibility may be even greater than usual. The cold treatment (DARLINGTON and LA COUR, 1940; GEITLER, 1940 a) or the unbalanced genotype due to hybridisation (KLINGSTEDT, 1941) can, for instance, cause the arising of differential segments.

It is probable that there are many other modifying factors the character of which is as yet unknown. It has, for instance, been impossible to distinguish between non-SAT-constrictions and differential segments in the investigations on the chromosome morphology. This is confirmed by the observations of the variation of secondary constrictions during the various stages of mitosis. SVÄRDSON (1945) has observed that in several *Salmo* species the number of constrictions is greatest at prophase and after that rapidly diminishes so that the constrictions are no longer visible and stain normally. This supports, as SVÄRDSON (l. c., p. 36) has also suggested, the above mentioned hypothesis of KLINGSTEDT concerning the character of allocycl. The morphological characters of constrictions can vary even during the same mitosis (CALLAN, 1942; cited after SVÄRDSON, 1945).

It has not been possible to find in the literature observations on the capability of differential segments to take part in the formation of nucleoli. *It seems, however, very probable that many, possibly all, heterochromites contain, at least as potential, the capability to give rise to nucleolar substance.* It would, therefore, be difficult to assume that the differential segment would make an exception.

References to chromosome bridges at anaphase not caused by dicentric chromatids (DARLINGTON, 1937) can be found quite frequently (see literature in TISCHLER, 1942, and DARLINGTON and UPCOTT, 1941). These bridges, which seem to be caused by the sticking together of parts of chromatids, have been named pseudo-pontes by RESENDE (1941), who suggested their cause to be chromatic agglutination. The best known term is, however, sticky phenomenon.

As the cause of stickiness BEADLE (1932, 1937) mentions a special gene (*st*) in *Zea*, KLINGSTEDT (1939) the hybridisation in grasshoppers, SVÄRDSON (1945) the same cause in Salmonids and LÖVE (1944) in *Rumex*. Among the chemical agents WITKUS and BERGER (1944) mention veratrine, ÖSTERGREN (1944 b) ethylene glycol, and LEVAN (1945) several inorganic salts. As other factors WHITE (1937) has observed

the X-ray radiation in grasshoppers and RESENDE (1941) in an *Aloë* hybrid, COUTINHO (1940; cited after RESENDE, 1941) a virus in *Vicia faba*. No external factors at all seem, according to RESENDE (l. c.), to be implicated in the sticky phenomenon in *Aloë mitriformis* var. *Commelinii*.

A statement of special interest is that of DARLINGTON and LA COUR (1940) concerning the coincidence of sticky phenomenon with differential segments. GEITLER (1940 b), CALLAN (1942) and WICKBOM (1945) comment on a similar tendency in their objects. The stickiness of the chromosomes may, therefore, in these cases be considered as caused by changes in the heterochromatin. KLINGSTEDT (1939) has explained the phenomenon as caused by a delay in the lapsing of the attraction of the sister-chromatids as compared with the onset of the anaphase movement. This is in accordance with the interpretation of DARLINGTON and LA COUR (1940) that the sticking tendency of the differential segments is due to a hindrance in the development of genes caused by nucleic acid starvation. The unreduplicated chromatids are, as a consequence, not able to separate. Later, however, DARLINGTON and UPCOTT (1941) have advanced another interpretation, viz. that the stickiness is due to the reunion of chromatids broken at earlier stages owing to cold treatment.

If against this background we consider the disturbances caused by ethyl alcohol and DDT + ethyl alcohol, we can observe that they are confined to metaphase. The other division stages including the resting stages are quite normal. As was stated earlier, the met-anaphasic abnormalities are: the appearance of metaphase nucleoli, the sticky phenomenon, the lack of matrix substance, and the spindle apparatus disturbance characteristic of c-mitosis. Among these partial effects the formation of metaphase nucleoli and sticky phenomenon has been suggested to be connected with the function of heterochromatin. The harmony in the collaboration of spindle apparatus and centromeres has a great significance for the continuance of the timing balance of mitosis. DARLINGTON (1937, p. 547) has expressed this thus: »The formation of the spindle and the successful orientation and division of the chromosomes therefore depend on a balance in the strength and timing of the cycles and repulsion of the centromeres and centrosomes». The disturbances in the normal timing relationships between several stages of division are very significant causes of division abnormalities. KLINGSTEDT (1939, p. 411) emphasizes the importance of them in his statement concerning DARLINGTON's precocity theory: »Without going

into the discussion which is still going on about the precocity theory as such, I would call attention to the general type of interpretation of which the precocity theory is a special case: I mean changes in the timing relationships of events, which play an important part in DARLINGTON's theories and will no doubt prove a valuable method of interpretation in biology».

When we examine the metaphasic abnormalities in *Allium* and *Trigonella* it is not possible to escape the impression that the disturbances just in the timing relationships are one of the most significant causes of them. *The abnormalities are strikingly reminiscent of conditions which, normally, are peculiar to telophase and resting stage.*

The diminishing of matrix substance is a process normally beginning at telophase and attaining its maximum at resting stage. In *Allium*, on which the most careful observations were possible, the lack of matrix substance already begins at prophase. At metaphase the chromosomes have the same external structure as at the end stages of telophase or resting stage, excluding the persisting spiral. Using the FEULGEN gentian-violet differential staining it is possible, just as at resting stage, to distinguish in the metaphase chromosomes the heterochromat as dark stained segments from the euchromatic parts. The observations of KOSTOFF (1938) on the metaphasic heterochromites in *Crepis capillaris* and *Triticum monococcum* are very similar. There is no allocyclus of heterochromatin here. The clearness of the spiral structure is exclusively due to the absence of the matrix that usually covers it. It cannot be analogous to the straightening of the chromonema spiral on treatment with several chemical agents, because the extra chromosome contraction exists simultaneously. The chromosomes of *Trigonella* have not been so successful objects for corresponding observations, but, nevertheless, it may be assumed that the same conditions prevail.

The formation of nucleoli is also a phenomenon normally occurring at telophase. It seems that in *Allium* as well as in a corresponding way in *Trigonella* the true nucleolar regions do not take any part in the forming of the metaphase nucleoli. These do not begin formal function until at telophase. It is clear that the heterochromatic segments of *Allium*, especially in the telomeric parts, begin to form nucleolar substance exceptionally and at exceptional time. That supports the earlier suggestion that not only the SAT-constrictions but also the many other heterochromites have a potential and in definite cases manifested capability to form nucleolar substance. A peculiar feature in this case

is the precocity of this manifestation compared with the normal nucleolar cycle.

The conditions observed in *Trigonella* deviate from those in *Allium*, the nucleolar substance not being formed as different bodies but entirely surrounding the metaphasic chromosome set. This is reminiscent of the meiosis of *Zea* described by McCLINTOCK (1934), where the nucleolus has arisen without any special nucleolar regions. On this ground one might conclude that *Trigonella*, except SAT-constrictions inactive at this stage, has no differentiated heterochromites, as *Allium*, capable of forming nucleoli at metaphase. The nucleolar substance might thus arise along the surface of the chromosomes. DERMEN (1933) considers this mode of developing as normal. Another and, as I think, a more possible alternative is that the nucleolus-forming capacity of *Trigonella* is relatively greater than that of *Allium* (the normal nucleolus—nucleus ratio is in *Trigonella* greater than in *Allium*). The substance formed on the heterochromites, therefore, quickly fills the spaces between the small chromosomes.

Metaphase nucleoli disappear before telophase in the two examined species. In *Allium* these often lag a long time, in *Trigonella* they are quickly dissolved in the cytoplasm. In both cases the nucleoli are not able to function further. As earlier mentioned, the metaphase nucleoli are never vacuolized. DERMEN (1933) has especially pointed out the significance of vacuolization as a criterion of functioning nucleolus. Because the nucleolar regions at telophase begin to form new, functioning nucleoli it has been suggested that *the metaphase nucleoli are not true nucleoli*. They are built up from a substance which probably would be identified with matter called by HEITZ (1931 a) n-substance and by RESENDE (1938) pre-nucleolar substance. This is raw material from which only the SAT-constrictions are able to form true nucleoli. Heterochromites accidentally producing pre-nucleolar substance have no such capability. It is important, however, to pay attention to the wholly different environment in which the metaphase nucleoli and true nucleoli have arisen. It seems conceivable that *the arising of true nucleoli presupposes a phase in which the nuclear sap surrounds the SAT-constrictions*.

The cause of the sticky phenomenon, the strong attraction of the distal heterochromites, indicates conditions prevailing at telophase and above all at the resting stage. As KLINGSTEDT (1941) has remarked, at the stages mentioned above heterochromites have a tendency to fuse and form bodies resembling nucleoli. For this reason it has been

suggested that *the sticky phenomenon is caused by precocity in the development of the heterochromites*. These have fused similarly as at resting stage, and we can show that the cause of attraction is the purely mechanical agglutination, as RESENDE (1941) also has pointed out. The interpretation given by DARLINGTON and LA COUR (1940) that the duplication of the chromonema is hindered by the lack of nucleic acid cannot be valid here because no starvation of nucleic acid has been observed. The later interpretation of DARLINGTON and UPCOTT (1941) also seems to be inappropriate, for it is difficult to assume a necessary number of chromatid breaks at earlier stages.

The synchronous disturbance of timing relationships at many stages of chromosome division is the chief results of the treatment described above. Abnormalities occur in the euchromatic as well as in the heterochromatic parts of the chromosomes. Metaphase chromosomes are actually under development at a stage corresponding to the telophase and resting stage. *As a real cause of the disturbance of timing relationships we must no doubt consider the c-mitotic disturbance of the spindle apparatus*. The chromosomes are in such a condition that the heterochromatic segments, but not the SAT-constrictions, begin to produce nucleolar substance. The substance formed constitutes the raw material of true nucleoli, so-called pre-nucleolar substance, and is later eliminated. The beginning of true nucleolus development presupposes that the SAT-constrictions are surrounded by nuclear sap. As a direct result of the precocity there also arises the sticky phenomenon with false bridges, although undoubtedly the agglutination of matrix caused by the used chemicals is also a very important factor.

Owing to the fact that the stickiness and simultaneously occurring metaphase nucleoli prevent the anaphase separation there are frequent breaks of the chromatids. It has been possible to observe this directly at anaphase of *Allium*. Especially the parts of chromatids fused with nucleoli are liable to become entirely eliminated. Since the poison effect of DDT + ethyl alcohol is relatively weak, the question should be investigated whether the effects at meiosis correspond to those observed at mitosis. BEADLE (1932, 1937) has observed a strong increase of the mutation rate in the progeny of maize plants affected by stickiness of the chromosomes.

It is an interesting feature, though not one that is possible to explain without further investigation, that the ethyl alcohol strengthens the effects of DDT. Are the described abnormalities the result of the co-action of the two chemical compounds? It seems, however, im-

probable that both these substances could have a similar specific effect. It is rather possible that only ethyl alcohol is likely to possess the property in question, DDT playing no direct part. Experiments with ethyl alcohol in combination with several other c-mitotically active substances could probably shed light on this problem.

In this connection I wish to extend my sincere gratitude to the Director of the State Horticultural Institute (Piikkiö, Finland), Prof. Dr. O. MEURMAN, for help and valuable criticism during my work in his institute. I am also very grateful to Mag. phil. EEVA SUOMALAINEN for her appreciated assistance. I am greatly indebted to Dr. A. LEVAN for valuable advice and fertile discussions during my stay at the Cytogenetic Laboratory of Svalöf Plant-Breeding Institute in the summer of 1945 and for all the ready help later given in the course of my work. I also thank Dr. G. ÖSTERGREN, who has read the manuscript and made valuable suggestions. In addition I wish to thank the Chemist of the Finnish Alcohol Monopoly, Dr. T. VOITILA, for the DDT substance kindly produced by him and Dr. V. KANERVO for supplying me with the literature concerning DDT.

### SUMMARY.

(1) An investigation is submitted of the effects of treating the root-tips of *Allium Cepa* and *Trigonella foenum graecum* with DDT insecticide (dichloro-diphenyl trichloro-methylmethane), ethyl alcohol and DDT + ethyl alcohol.

(2) The combined FEULGEN and iodine-gentian-violet differential stain was used to distinguish nucleolus and chromatin. In objects treated with ethyl alcohol and DDT + ethyl alcohol this gave a satisfactory result.

(3) Pure DDT substance (saturated tap-water solutions) was observed to have a weak c-mitotic effect. The extra contraction of metaphasic chromosomes was measured.

(4) In *Allium Cepa* treatment with pure ethyl alcohol (3,3—13,3 vol.% tap-water solutions) caused, besides the extra chromosome contraction, the effects characteristic of partial c-mitosis. It was observed that, in the heterochromatic telomere parts, metaphase chromosomes produce nucleolar bodies which are dissolved in the plasma during anaphase. The matrix around the chromonemata is weakly developed.

The structure of the chromonema is quite clearly visible. At anaphase the sticky phenomenon can be seen, i. e. the tendency of the distal ends of chromosomes to fuse and thus form false bridges.

The proper nucleolar regions of the chromosomes, SAT-constrictions, do not take part in the formation of metaphase nucleoli but function normally. Hence the cycle of the true nucleoli is regular. The metaphase nucleoli are not residues of prophasic true nucleoli but are new formations that have arisen after prophase.

In *Trigonella* a small amount of nucleolar substance is produced on the surface of the chromosomes by treatment with ethyl alcohol. Sticky phenomenon does not occur. Metaphase figures are normal.

(5) Using DDT + ethyl alcohol (saturated DDT solution + 3,3—13,3 vol.% ethyl alcohol), the same effects as with ethyl alcohol series become visible, though they are more powerful and frequent.

In *Allium*, besides the above mentioned c-mitotic chromosome contraction, multipolar spindles and in some cases polyploid cells also occur. Metaphase nucleoli are not wholly resorbed in the plasm but remain as laggards between the sister-nuclei. Chromatid breaks and small chromatin eliminations also take place owing to the stickiness and the hindering effect of the lagging nucleoli on the anaphase separation.

In *Trigonella* the metaphasic nucleolar substance entirely covers the chromosomes. This substance coating the chromosomes does not give rise to lagging nucleolar bodies. The substance disappeared before the beginning of telophase. Sticky phenomenon is intensive. Metaphase orientation seems to be regular.

(6) When the onions treated with DDT + ethyl alcohol are transferred to pure tap-water, even after two days the same mitotic abnormalities as in root-tips fixed at the DDT + alcohol treatment can be observed.

(7) The current literature concerning the development of the above-mentioned mitosis abnormalities has been studied. The conclusion can be drawn that functional similarities can be found between the SAT-constrictions, non-SAT-constrictions and the heterochromatic regions of chromosomes called differential segments. These heterochromites have, if not manifestly yet at all events potentially, the capability of forming nucleolar substance.

(8) It is suggested that, besides the specific effect of ethyl alcohol and DDT, the disturbance of timing relationships between the different stages of the mitotic chromosome cycle is one of the most important

causes of the described mitotic abnormalities. The disturbance is caused by the c-mitotic narcosis of the spindle apparatus and the consequently retarded division. Euchromatic as well as heterochromatic parts of chromosomes are at metaphase already in a developing phase corresponding to telophase and resting stage. In normal conditions the disturbances or the direct consequences of them are characteristic phenomena of the above-mentioned stages.

(9) It has also been suggested that the metaphase nucleoli consist of pre-nucleolar substance, i. e. of the raw material of true nucleoli, and are not, therefore, able to function. In the author's opinion the true nucleoli cannot as a rule arise elsewhere than in connection with SAT-constrictions when these are surrounded by nuclear sap. The true nucleoli cannot here be formed from this pre-nucleolar substance which is thus excluded from the normal nucleolar cycle.

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# INVESTIGATIONS ON C-MITOSIS IN ALLIUM CEPA

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## I. THE CYTOLOGICAL EFFECT OF HEXACHLORO- CYCLOHEXANE.

SINCE the discovery of the well-known cytological effect of colchicine many other organic compounds have been shown to have the same effect on mitosis. Among other investigators, LEVAN and ÖSTERGREN (1943) and ÖSTERGREN and LEVAN (1943) found c-mitotical activity in about 30 substances, and in 1944 ÖSTERGREN tested about 50 other substances, most of which proved to possess the same properties. The works of LEVAN and ÖSTERGREN also led to the discovery of the principle that the activity of many c-mitotic agents is conditioned not so much by their chemical properties as especially by their physical ones, and this in turn caused them to propound their theory of the narcotic nature of the c-mitosis.

It seemed interesting to us to study the ability of the well-known insecticide hexachlorocyclohexane to induce c-mitosis. Four different isomers of the substance are known, and they are all well separated as regards both their physical properties and their insecticidal effects (cf. SLADE, 1945 a and b). Three pure isomers have been available to us, viz. the  $\alpha$ -,  $\beta$ - and  $\gamma$ -isomers, as also the crude product »666». The composition of »666» is as follows:  $\alpha$ -isomer 10—12 %,  $\beta$ -isomer 70 %,  $\gamma$ -isomer 5 %,  $\delta$ -isomer 10 %, together with some chlorinated compounds of an unknown nature.

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The investigations have been carried out at the Cyto-genetic Laboratory, Svalöf, and at the Institute of Genetics, Lund. Our thanks are due to the Chief of the former Institute, Dr. A. LEVAN, as also to Dr. G. ÖSTERGREN, Lund, for their valuable advice and for discussing the problems. We are also very much indebted to A.-B. Pharmacia (Mr. E. ASKELÖF), Stockholm, and A.-B. Astra (Dr. S. WIEDLING), Södertälje, for their ready courtesy in providing us with the substances needed.

## 1. METHODS.

In the main the methods used were the same as those employed earlier by LEVAN and ÖSTERGREN (1943). To get exact concentrations of the substances we first made concentrated alcohol solutions, which were then diluted with water (tap-water). The strongest concentration of all isomers made in this way contained 0,07 % ethyl alcohol, which, however, could hardly have had any effect of interest in our case. As material for the treatments we used bulbs of *Allium Cepa*, the roots of which were immersed in the various solutions. The root tips were fixed in Navashin and, after embedding, cut into longitudinal sections and stained with crystal violet.

## 2. DESCRIPTION OF THE RESULTS.

At the study of the cytological effects there appeared some very interesting differences between the isomers. The results of the treatments are collected in Table 1. Beginning with the c-mitosis, the most insecticidal  $\gamma$ -isomer proved to be able to induce complete c-mitosis in concentrations of 0,000·01 mol/litre and more. In 0,000·005 mol/litre there occurred multipolar anaphases indicating spindle disturbances, partial c-mitosis. The complete c-mitosis was quite typical and agreed with that described, e. g., by LEVAN (1938). On the other hand, we never observed any tendencies to such a distribution of the chromosomes as is obtained after treatment with methylnaphthoquinone.

The next most insecticidal  $\alpha$ -isomer also brought about spindle disturbances, partial c-mitosis, but was unable to induce complete c-mitosis even in saturated solutions. The c-mitotical efficiency of this isomer was thus much weaker than that of the  $\gamma$ -isomer.

Concerning the third, the least insecticidal  $\beta$ -isomer, it proved to be quite without any c-mitotical activity, even saturated solutions being unable to produce any disturbances.

The mixture of the various isomers, »666«, induced complete c-mitosis, probably owing to its content of  $\gamma$ -isomer, an assumption supported by the situation of the threshold value. The  $\delta$ -isomer, not tested by us, may have had some effect as well.

In tests on the ability of the saturated solutions to induce c-tumours the  $\gamma$ -isomer was also found to differ from the other isomers, being alone able to produce this effect.

No poison effect was obtained in any solutions after 24 hours' treatment, but after 48 hours the roots were quite slack and obviously

TABLE 1. *The various properties of the isomers of hexachlorocyclohexane and the crude product »666«.*

+ = presence and — = absence of the effect in question.

Substances:		$\gamma$	$\alpha$	$\beta$	»666«
The effects:					
C-mitosis	0,000'1	+	±	—	+
	0,000'05	+	±	—	+
	0,000'02	+	±	—	±
	after 4 hours 0,000'01	+	±	—	—
	0,000'005	±	—	—	—
Conc. in mol/litre	0,000'002	—	—	—	—
	0,000'001	—	—	—	—
C-tumours after 24 hours Saturated solutions		+	—	—	+
Poison effect (death of roots) Saturated solutions					
After 24 hours		—	—	—	—
After 48 — 200 hours		—	—	—	+
Rel. insecticidity <i>Meligethes</i> (frequency of dead beetles in % of that of the $\gamma$ -isomer)		100	94,3	70,7	87,9
Rel. insecticidity <i>Colandria</i> (after SLADE, low value = high insect.)		1	900	inactive	
Melting point (after SLADE)		112,5°	158°	309°	
Water solubility in 10 <sup>-6</sup> mol/litre		100— —200	50— —100	20— —50	100— —200

dead in saturated solution of »666«. The  $\gamma$ -isomer, although very poisonous to insects, showed no signs of such toxic effects as killing of the roots even after long and repeated treatments with saturated solutions.

To get acquainted with the insecticidal effects of the various isomers of hexachlorocyclohexane we performed some experiments to this end, using the same concentrations of the isomers as used for the c-mitotical treatments.

Turnip blossom beetles (*Meligethes aeneus* F.) were put into covered Petri dishes at the bottoms of which there were filter papers moistened with 1 c. c. of the solutions. After three hours the beetles were counted, and the percentage of dead beetles was determined. These values are represented in Diagram 1. In the main our results coincide with earlier results of other authors. The  $\gamma$ -isomer proved to be the most insecticidal one,  $\alpha$ - the next, and finally the  $\beta$ -isomer the least insecticidal one. The effect of »666» was intermediate.

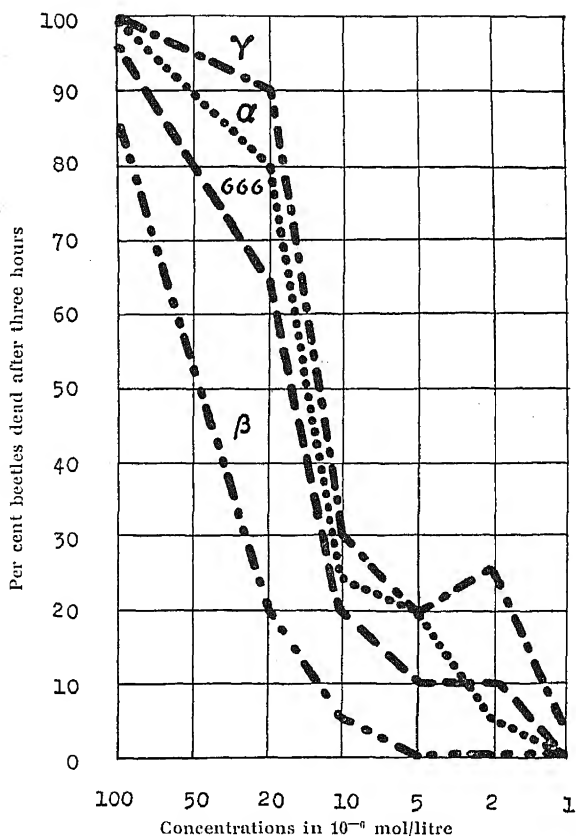


Diagram 1. The insecticidal effects of the various isomers of hexachlorocyclohexane and the crude product »666». Concerning data, see text.

### 3. DISCUSSION.

Insecticides may perform their action in three ways, viz. as contact agents, as fumigants, or as stomach poisons. LÄUGER, MARTIN and

MÜLLER (1944) consider lipoid solubility to be conditional upon the action as contact agent. The cuticle of the insects, among other things, contains lipoids by which the contact agent is supposed to penetrate. A fumigant insecticide must be volatile, and the physical properties of the stomach poisons, finally, may be widely varying.

While certain insecticides, as e. g. the well-known DDT, have a specific contact activity, »gammexane» (the  $\gamma$ -isomer of hexachlorocyclohexane) by SLADE (1945) is said to be active in all the three ways. Judging from the investigations of SYLVÉN (1947), its action as a fumigant seems to be very important, and the experiences of SLADE point in the same direction.

As regards the mode of action of hexachlorocyclohexane in a physiological respect, it is close at hand to assume that it is acting as a narcotic. Unfortunately no literature on this aspect of the action of gammexane is available to us. Other chlorohydrocarbons, as e. g. chloroform and carbon tetrachloride, being also insecticides, are known as narcotics, and they probably produce their insecticidal effects as narcotics. As is well known, too strong doses of narcotics have a toxic effect, killing the cells instead of producing a reversible narcosis.

According to the classical MEYER-OVERTON theory of narcosis, the narcotics produce their action by dissolving in the lipoids of the cells, and lipoid solubility is usually considered as an important condition of narcotic activity.

If we consider the physical and insecticidal properties of the different isomers of hexachlorocyclohexane, we shall find a connection between low melting point, high water solubility and insecticidity (cf. Table 1). The differences in melting point should undoubtedly also be connected with differences in solubility in most solvents, among them also lipoids, viz. in such a way that low melting point entails a high solubility.

If the insecticidity of these substances is conditioned by their narcotic properties, a connection was to be expected between this insecticidity and the lipoid solubility of the substances. Owing to its relatively low melting point and undoubtedly relatively high lipoid solubility, the  $\gamma$ -isomer should be expected to have the strongest insecticidal effect.

The rather small differences between the isomers in our insecticidity trial are probably referable to our methods; among other things the gas concentrations were very high in our covered Petri dishes.

} Concerning the mechanism of the c-mitotic action, there are in the

main two theories. In 1943 LEVAN and ÖSTERGREN for the mechanism of the c-mitosis proposed a kind of lipoid theory corresponding to the MEYER-OVERTON theory of narcosis and, thus, »the decisive concentration of the substance may be that in the lipoids not in the water phase of the cells». This assumption explains the connection between the solubility and c-mitotical activity which these authors had found to be characteristic of the substances studied. Furthermore, they supposed that the c-mitosis was simply to be considered as a narcosis of certain enzymic functions of the cells. Even the c-tumour effect was considered as a narcosis of the growth control of the cells.

In 1944 ÖSTERGREN had arrived at a somewhat modified theory. He lays stress upon the view that the c-mitosis is to be considered simply as a narcotized cell division. The spindle breakdown characteristic of the c-mitosis he supposes to be caused by the fibrous components of the spindle being brought to assume a more or less corpuscular shape by the action of the c-mitotic agent, and this in turn would lead to the disorganization of the spindle.

Both these theories agree with the connection that seems to exist between the c-mitotic activity of the substances and their solubility relations, viz. a positive correlation between activity and lipid solubility and a negative correlation between activity and water solubility. ✓

If we put these considerations into connection with the results of our experiments, we shall find that the differences between the isomers may be quite explicable. The  $\gamma$ -isomer has a relatively low melting point and certainly also relatively high lipid solubility. Its higher c-mitotic activity and its ability to induce c-tumours are, therefore, completely expected, as was also its insecticidal effects. The  $\alpha$ -isomer has a higher melting point and lower solubility, and its concentration in the lipoids may be supposed to be just at the threshold of c-mitosis and under that of c-tumours. The  $\beta$ -isomer, finally, has the highest melting point and the lowest solubility and is for that reason quite without any c-mitotical activity.

As already mentioned, we could not get the  $\delta$ -isomer. As its melting point (138—139° C.) is between those of the  $\gamma$ - and  $\alpha$ -isomers, it might be expected to possess a certain degree of c-mitotic activity.

The differences found by us between the different isomers of hexachlorocyclohexane are completely analogous to those found by LEVAN and ÖSTERGREN (1943) to exist between the  $\alpha$ - and  $\beta$ -naphthalene derivatives. The  $\alpha$ -derivatives studied were all able to induce c-mitosis, whereas the corresponding  $\beta$ -derivatives were characterized by higher

melting points, lower solubility as also by a weaker c-mitotic efficiency.

Regarding the poison effect on the *Allium* roots, its nature sometimes seems to be more unclear, even if it, according to ÖSTERGREN (1944), in many cases may be a narcotic effect too, in such cases probably in some way analogous to the insecticidal effects of certain narcotics. In other cases the poison effect is certainly of another nature than narcotic, especially when it is brought about by substances with high water solubility and low lipid solubility.

As seen from Table 1, the pure isomers of hexachlorocyclohexane are quite without any poison effects and did not kill the *Allium* roots, whereas saturated solutions of »666» killed the roots after 48 hours' treatment. The poison effect of this crude product is probably caused by some impurities in this substance. We also made some orientating studies with an ether extract of a commercial mixture containing »gammexane». It proved to be able to induce c-mitosis, but it also had a poison effect similar to that of »666», and even in this case probably caused by impurities, e. g., chlorinated decomposition products.

According to LEVAN and ÖSTERGREN (1943), the practical applicability of a substance for the production of polyploids is largely dependent on its lack of toxic properties and its range of active concentrations, i. e. the relation between its solubility and threshold value.

Next to colchicine, which is unique in several respects, acenaphthene is the substance that is responsible for the greatest number of chemically induced polyploids, this depending not only on the fact that it has been in use for a long time, but also that it has proved not to be poisonous.

It does not seem improbable to us that even the  $\gamma$ -hexachlorocyclohexane might be useful for this purpose. In similarity to acenaphthene it is not poisonous, but in addition its range of active concentrations is much wider. The relation between solubility and threshold concentration is for  $\gamma$ -hexachlorocyclohexane ca. 30, whereas the same value for acenaphthene is only ca. 4. Judging from our experiences, however, purity seems to be necessary to get rid of the poison effect.

*Summary.* — Three pure isomers and a crude product of the well-known insecticide hexachlorocyclohexane have been studied as regards their cytological effects on *Allium* roots, especially their ability to induce c-mitosis.

The crude product as well as the most insecticidal  $\gamma$ -isomer proved to be able to induce complete c-mitosis, whereas the  $\alpha$ -isomer only

induced partial c-mitosis and the  $\beta$ -isomer was quite without any c-mitotical properties.

There may be a connection between insecticidity, c-mitotical activity and the simple physical properties of the substances, which is in complete agreement with the theory of the narcotic nature of c-mitosis.

As the  $\gamma$ -isomer was quite without any toxic effects on the *Allium* roots and at the same time was able to induce c-mitosis, it might be practically applicable for the production of polyploids.

## II. THE CYTOLOGICAL EFFECT OF VITAMIN K.

When studying the cytological effect of various organic compounds we investigated, among other things, the ability of three synthetical types of vitamin K to induce c-mitosis.

The substances used were: 2-methylnaphthoquinone, 2-methyl 1—4-naphthohydroquinone diacetate and sodium 2-methylnaphthohydroquinone 1—4-diphosphate. In spite of their simpler chemical constitution these substances have the same medico-biological effect as natural vitamin K, viz. to enable the coagulation of blood by coöperating at the formation of prothrombin (cf. MÖLLER, 1943).

### 1. METHODS.

The methods were the same as used when studying the cytological effect of hexachlorocyclohexane. As material for the treatments we used bulbs of *Allium Cepa*, the roots of which were immersed in the various solutions of the substances.

The methyl-naphthohydroquinone diacetate and sodium-methyl-naphthohydroquinone diphosphate were pure products from A. B. Astra. Under the name of »K-vimin» these substances have pharmacological application, the acetate as an oil solution and the phosphate as pills.

### 2. DESCRIPTION OF THE RESULTS.

*Methylnaphthoquinone*. — The results of the treatments are collected in Table 2. The solubility of the substance seems to be about 0,000·5 mol/litre, and it is, thus, to be considered as only very sparingly soluble in water.

In concentrations of 0,000·1 mol/litre and more we obtained typical c-mitoses after four hours' treatment. C-tumours did not appear, obviously due to the short time of treatment. The highest concentrations had a very marked poison effect resulting in pycnotic, degenerating



nuclei, and even in  $0,000.2$  molar solution there occurred some pycnotic nuclei, especially in the outer cell layers. Consequently, the roots in the two strongest concentrations showed reduced turgescence.

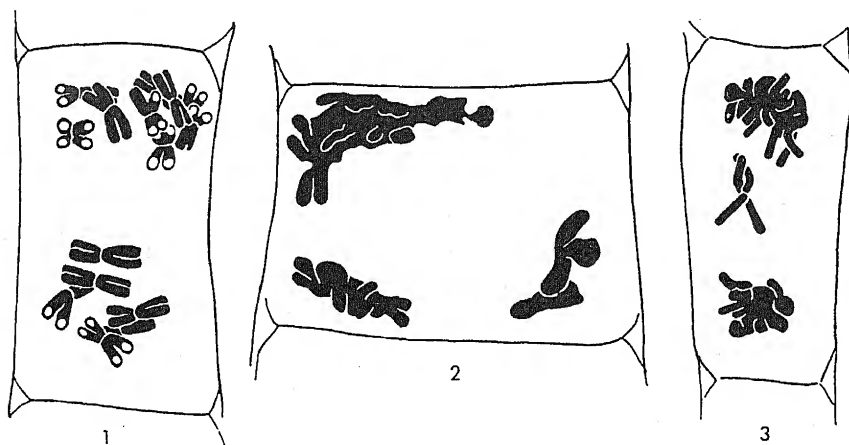
Probably the agglutination of the chromosomes appearing in the same solutions is to be considered as a kind of poison effect too. It expresses itself in the chromosomes becoming liquefied and baked together to an irregular lump. Such agglutination is certainly identical with the »sticky chromosome» effect, several cases of which are known, e. g., after treatment with some other c-mitotically active substances, e. g. ethylene glycol (ÖSTERGREN, 1944). In our case, however, the formation of anaphase bridges due to stickiness is rendered impossible by the lack of spindle activity. In ethylene glycol the threshold concentration of agglutination and stickiness is lower than that of spindle destruction.

After 24 hours' treatment there occurred c-tumours in those of the strongest concentrations in which the toxic action did not hinder the production of this effect. Now the poison effect itself was very marked also in weaker solutions, and even in  $0,000.1$ -molar solution the roots were partly dead, resulting in lack or reduction of turgescence. While the threshold of poison effect had thus been lowered, the threshold of agglutination and c-mitosis had been raised about one step in the concentration series, and these effects were now obtained only in solutions that were at the same time more or less poisonous. The c-mitosis was obtained only in  $0,000.2$ -molar solution. This c-mitosis differed, however, strikingly from the typical one described, e. g., by LEVAN (1938) and SHIMAMURA (1938). Instead of lying scattered in the cells, dividing and forming telophase nuclei, usually one per cell, the undivided chromosomes (c-pairs) are in this case of ours distributed at random to the two poles of the cells, where they divide and are usually inclosed in two telophase nuclei.

At first sight this telophase is similar to a normal telophase, but it differs from this in several respects conditioned by the absence of a normal spindle mechanism in these cells, the distribution of the c-pairs being brought about by some other forces. Firstly, the distribution of the chromosomes is obviously quite at random, sometimes resulting in the formation of »daughter» nuclei of very unequal size. Furthermore, these nuclei are without that polarization of centromeres and chromosome arms typical of normal telophase nuclei, and, finally, they are not separated by a cell-wall, this defect being also caused by the lack of spindle.

When this type of c-mitosis, which we term »*distributed c-mitosis*», is strongly pronounced, the c-pairs seem to be pressed against the end-walls of the cells by some force from its centre. Sometimes there occur not only two but several groups of chromosomes, these lying more or less close to the cell-walls, and the telophase picture is then very much like the »*exploded metaphase*» described by BARBER and CALLAN (1943).

Occasionally there may also lie a distinct group in the centre of the cells in addition to the polar ones.



Figs. 1—3. Distributed c-mitoses at anaphase and transitions to telophase.

Besides this distributed c-mitosis, there also occur quite typical ones, without distribution of the chromosomes, as also transitions between them, but the type described is much too predominating to be merely a fortuitous phenomenon. No tendencies to such distribution of the chromosomes were seen in any other concentration or after only four hours' treatment.

The chromosomes were contracted to about half their normal length, and their shape was the same as at typical c-mitosis. The late division of the centromeres was very characteristic. Neither spindle fibres in the plasma nor any other signs of spindle activity could be detected.

*Methyl-naphthohydroquinone diacetate.* — The water solubility of this substance too is rather low, 0,001—0,000·5 mol/litre. It proved to be unable to induce complete c-mitosis even in saturated solutions. These solutions, however, possessed a certain degree of c-mitotical activity that

gave rise to multipolar mitoses, regarded as partial c-mitoses by ÖSTERGREN (1944).

After 24 hours' treatment the saturated solutions showed a strong poison effect. No c-tumours were obtained in any solutions.

*Sodium-methylnaphthohydroquinone diphosphate.* — As might be expected from its chemical constitution, the water solubility of this substance is very high. We did not determine the solubility exactly, but even a 1-molar solution was without a precipitate. As seen from the table, this substance is quite devoid of any c-mitotical activity. Not even a 0.1-molar solution was able to bring about any c-mitotical disturbances. The only effects obtained were poison effects after 24 hours' treatment, and tendencies to agglutination of the chromosomes, but these effects probably do not belong to the c-mitotical complex of reactions.

### 3. DISCUSSION.

As to the differences in c-mitotical activity between the substances tested, we would suggest the following explanation.

The works on c-mitosis by LEVAN and ÖSTERGREN undoubtedly show that in many cases the activity of the c-mitotical agents is mainly conditioned by their physical properties, especially their solubility relations. These authors have found that there is a negative correlation between water solubility and c-mitotical activity, and this correlation is explained on the assumption that the substances produce their action dissolved in the lipoids of the cells, and thus the lipid solubility is of decisive importance.

The sodium-methylnaphthohydroquinone diphosphate is a salt and should therefore hardly be perceptibly soluble in lipoids. Its c-mitotical inactivity is, thus, very well expected. It can never produce its action in the same way as the methylnaphthoquinone, which must be assumed to have at least a certain degree of lipid solubility and to be able to induce c-mitosis. Concerning the acetate, this substance may also be expected to be somewhat soluble in lipoids and should consequently also be expected to give c-mitosis. It is difficult, however, at present to say whether it should be more or less active than the methylnaphthoquinone, the chemical structure being too different.

It has been more difficult to find a plausible explanation of the origin of the »distributed c-mitosis». Judging from their figures and description, WITKUS and BERGER (1944) seem to have obtained a most similar or even identical deviation from the typical course of c-mitosis

in their »pseudo-anaphase». These authors are of the opinion that there is an imperfect spindle active, but they do not mention anything as to the nature of this spindle. Besides, the term »pseudo-anaphase» is perhaps somewhat inadequate, as typical c-chromosomes also pass through an anaphase and a telophase.

Above it was mentioned that the distributed c-mitosis is sometimes very similar to the »exploded metaphase» obtained by BARBER and CALLAN (1943) in *Triton* after treatment with cold and colchicine. These authors explain its origin in the following way: »In the exploded anaphase the chromosomes are pushed bodily about the cell, probably owing to the formation of a purely centrosome spindle.» The centromeres are considered to be inactive and the c-pairs are pushed about the cell just as at our distributed c-mitosis.

Making use of ÖSTERGREN's (1945) theory concerning the existence of certain transverse forces in the spindle tactoid which act on the chromosome bodies, we are venturing to put forward an explanation of the origin of our distributed c-mitosis, one that is perhaps also applicable to the »pseudo-anaphase» and the »exploded metaphase».

It does not seem impossible to us that the forces acting on the c-pairs might be of the same kind as those proposed by ÖSTERGREN, in normal cells »manifested as tendencies of the chromatids to move out of the spindle» and resulting in the origin of »transverse equilibria on the spindle». It may be suggested that in spite of the absence of any visible spindle mechanism there is still a kind of coacervate or tactoid, the particles of which push the chromosomes in a peripheral direction, thus causing the characteristic distribution of the chromosomes described by us.

In fact, observations by other authors indicate that at c-mitosis there exists an element in the cells that might produce such forces, viz. the achromatic sphere observed by SHIMAMURA (1938) and BERGER and WITKUS (1943) around which the chromosomes sometimes seem to arrange themselves.

The increased threshold concentration of c-mitosis after 24 hours' treatment is also very difficult to interpret. Possibly the cells have a certain ability of getting inured to the treatment. Regarding the camphor reaction of yeast, LEVAN and SANDWALL (1943) state that »the cells may gradually become insusceptible to the substances» during the treatment, whereas ÖSTERGREN (1944) in *Allium* has in some cases observed a »downward movement of the c-mitotic threshold».

*Summary.* — Three pharmacologically used types of vitamin K are tested as regards their ability of inducing c-mitosis in root-tip cells of *Allium*. Methyl-naphthoquinone proved to be able to induce complete c-mitosis, whereas methyl-naphthohydroquinone diacetate only induced partial c-mitosis and sodium-methylnaphthohydroquinone diphosphate was quite without any c-mitotical activity.

The differences in activity agree with the opinion of LEVAN and ÖSTERGREN that the solubility properties are of decisive importance for the c-mitotical activity of organic compounds.

There is also described a peculiar type of c-mitosis that deviates from the typical c-mitosis by having the undivided c-pairs distributed to the two poles of the cells.

An explanation of the origin of this »distributed c-mitosis» is submitted.

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# CONTRIBUTIONS TO A CYTOLOGICAL ANALYSIS OF THE SPECIES DIFFERENCES OF *GODETIA AMOENA* AND *G. WHITNEYI*

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THE parallel genetical and cytological investigations of the *Godetia* material in the cultures of Dr. HIORTH have given certain results that also seem to be of interest to students of the problem of speciation. Such a result was the production of a *G. Whitneyi* with the new basic number 6, previously unknown in the genus *Godetia* (HIORTH, 1946 c; HÅKANSSON, 1946). The new form had been produced through intra-specific crosses between different monosomics. In this paper the behaviour of the chromosomes in repeated backcrosses of the interspecific hybrid *G. amoena*  $\times$  *G. Whitneyi* to *G. Whitneyi* will be described, and also some cases of spontaneous chromosome changes.

HIORTH (1942) has shown that *G. amoena* occurs only in a rather small area south of Golden Gate, while *G. Whitneyi* has a wider distribution from Golden Gate to the Vancouver Island in British Columbia. The most important morphological species difference consists of certain patterns on the petals. *G. amoena* has a basal spot quite at the base of the petal; such a spot never occurs in *Whitneyi*. *G. Whitneyi* has a central spot of varying size nearer the centre of the petal; this spot occurs in some garden races of *amoena* but never in *amoena* plants from natural localities. Individuals lacking petal spots may occur in the two species. HIORTH (1940) has shown that the spotless condition is recessive (*f*), that there are several central spot alleles, each causing a different size of this spot ( $F^x$ ), and that the basal spot gene of the *amoena* race used in the crosses and designated  $F^b$  is allelic to  $F^x$  and *f*. This allelism of  $F^b$  and  $F^x$  has been fully confirmed by the results of later crosses of HIORTH. Its manifestation is perhaps unusual;  $F^bF^x$  plants have both a basal and a central spot. Earlier, RASMUSON (1921) had studied the genetics of these petal spots and found that »a case of multiple allelomorphism possibly is present here» (l. c., p. 284).

The question of the true nature of the species barrier has been studied by HIORTH, who used the method of attempting to transfer

certain important species characters of *amoena* to *Whitneyi*, first backcrossing the  $F_1$  hybrid to *Whitneyi*, and then through successive generations crossing plants with the character studied to *Whitneyi*. Great difficulties have met the production of fertile *Whitneyi* plants having  $F^b$  (HIORTH, 1946 b).

*Godetia amoena*  $\times$  *G. Whitneyi* is very sterile. In the first backcross generation,  $F'_2$ , all  $F^b$  plants were very sterile, while at least some  $F^x$  plants were fertile. Also in  $F'_3$  all  $F^b$  plants had been very sterile. In the 3rd and following backcross generations certain distinctive  $F^b$  types appeared, the properties of which will be discussed in the following pages. As a rule,  $F^b$  plants also showed a pronounced pollen and seed sterility in the later generations, whereas  $F^x$  and  $f$  plants were fertile. Selfing of the  $F^b$  plants or crossings with these plants gave a low number of  $F^b$  plants,  $F^x$  or  $f$  plants appearing in a great excess. Thus the transfer of  $F^b$  to *G. Whitneyi* has met with great difficulties, the  $F^b$  plants in the crosses being more or less sterile and segregating only a low percentage of  $F^b$ . However, a fertile  $F^b$  with a more normal segregation has at last appeared.

Many  $F^b$  plants from the first and later backcross generations have been investigated cytologically, and my results are mentioned in the communication of HIORTH, but an illustrated description of the cytological observations is needed. Buds have been fixed (in Ås) of: (1) *amoena*  $\times$  *Whitneyi*  $F_1$  in 1941, (2)  $F_1 \times$  *Whitneyi*, the first backcross generation, designated  $F'_2$  in 1943, (3) the 3rd backcross generation  $F'_4$  in 1941, (4) the 4th and 5th backcross generations  $F'_5$  and  $F'_6$ , belonging to the same pedigree as  $F'_4$ , in 1942 and 1943. The genes involved in these crosses were  $F^b$ — $F^x$ — $f$  and  $W'_1$ — $w_1$ .  $W_1$  is one of three genes that cause a light violet instead of a white colour of the petals.  $F^x$  and  $W_1$  in *G. Whitneyi* show linkage with crossing-over values of more than 20 %. The *Whitneyi* chromosome having these genes has been designated 1.2 (HIORTH, 1946 c).  $W_1$  from *amoena* was called  $W'_1$ .

*Godetia amoena*  $\times$  *G. Whitneyi*,  $F_1$ . — Meiosis has been described several times. The hybrid is very sterile. HIORTH found a seed fertility of about 3 % and a pollen sterility of 94 %. The chromosome pairing is, however, good; the most common number of I's is 2. The hybrids investigated in 1931 showed III's, IV or a chain-of-five (HÅKANSSON, 1931); in *G. amoena* Sunol  $\times$  *G. Whitneyi* Bremen no multivalents seemed to occur (HÅKANSSON, 1942). In a re-investigation of the latter hybrid, however, multivalents were detected. As is shown in Fig. 1 a,

at least 2 configurations-of-three may be formed. One is a heterochain with a rather small end-chromosome, the other is a homochain of about equal chromosomes. Sometimes there are 7 II; it seemed as though the small chromosome was paired with a chromosome from the latter configuration (Fig. 1 *b*). Often the small chromosome is univalent, the heterochain being instabile. The second configuration-of-three is more often observed. Rarely, a III with a triple chiasma is seen (Fig. 1 *c*), though it is uncertain whether this is a third configuration-of-three. An interstitial chiasma is sometimes observed in the chains (Fig. 1 *d*). Some p.m.c.'s with 5 II + chain-of-four were observed and very rarely a closed ring-of-four (Fig. 1 *e*). The configuration-of-four is more rare than the second configuration-of-three and, as Fig. 1 *f* shows, these two configurations may occur together. Another high catenation was chain-of-five. The structural differences between the standard genome of *Whitneyi* and *amoena* are also shown by the occurrence of unequal II's (2 or 3); very rarely is an inversion bridge seen. Fig. 1 *g* shows a somatic plate from a petal cell and gives an impression of the size relations of the chromosomes of the  $F_1$  hybrid.

The occurrence of multivalents shows reciprocal translocations and duplications in the chromosomes. However, many p.m.c.'s have no multivalent, and »homologous» segments are thus often unpaired. Probably no p.m.c. was observed where all possible pairings were realized. HIORTH thinks that such a pairing would be ring-of-six + ring-of-four + two II's. A cytological demonstration does not seem to be possible, perhaps owing to this »asyndesis». In view of the strong structural differences and the absence of a large ring or chain that could bring about a regular assortment of the chromosomes, the high sterility of this hybrid is not surprising.

$F_1 \times G. Whitneyi$ . — The cross investigated had been of the type  $(F^b W'_1 \times F^x w_1) \times f w_1$  and the offspring  $1 F^b F^x W'_1 : 22 F^b W'_1 : 5 F^b w_1 : 12 F^x W'_1 : 36 F^x w_1$ . As HIORTH points out, crossing-over had been as high as in intraspecific crosses of *G. Whitneyi*. Buds from rather many plants had been fixed; the pollen fertility of these plants had been determined by HIORTH. Four  $F^x$  plants were investigated. They were all cross-overs ( $F^x W'_1$ ) and were pollen-fertile, having a pollen sterility of only 6 %. They formed 7 II and had a regular meiosis. No  $F^x w_1$  had been fixed or had its pollen investigated. Some of them must have been sterile (p. 241). In mode of growth all  $F^x$  plants were similar to *Whitneyi*.

All  $F^b$  plants in the cross had a high pollen sterility, some had 70—

80 %, others had more than 90 % poor pollen. Only one of them had the mode of growth of *Whitneyi*, the rest were more similar to *amoena* in this respect. No  $F^b$  had regularly 7 II and a regular meiosis; all investigated plants (19) showed the presence of *amoena* chromosomes.

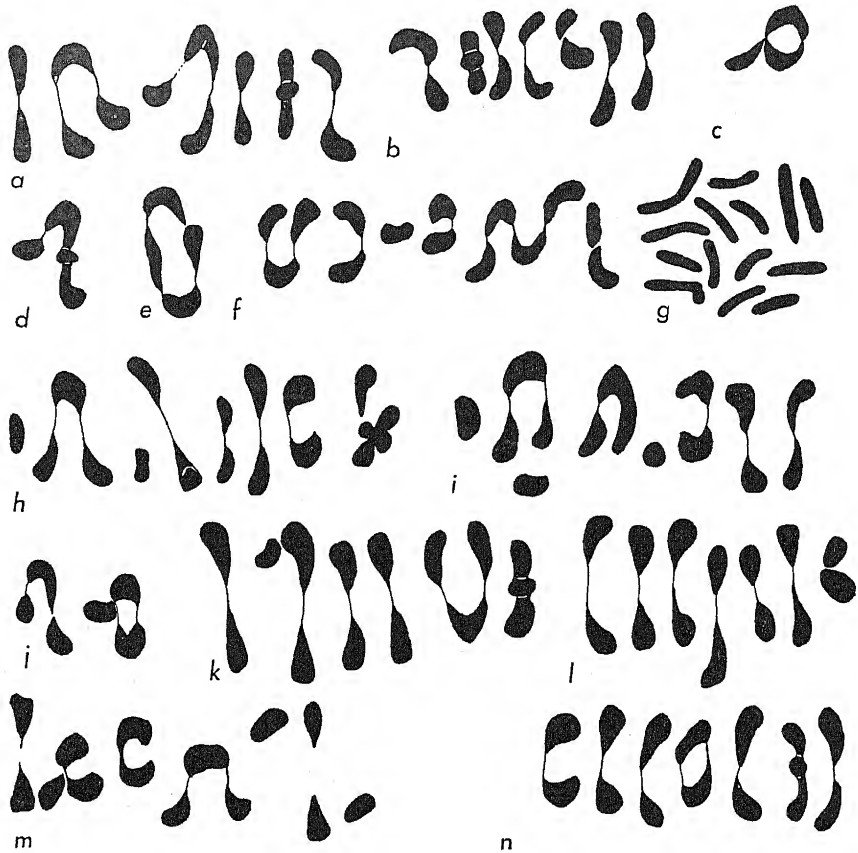


Fig. 1. *a-g*. *G. amoena*  $\times$  *G. Whitneyi*. — *a*: 4 II + homo-III + hetero-III. — *b*: 7 II. — *c*: configuration with a triple chiasma. — *d*: heterochain showing an interstitial chiasma. — *e*: ring-of-four. — *f*: IV + III + 2 II + 3 I. — *g*: somatic plate. — *h-n*. (*G. amoena*  $\times$  *G. Whitneyi*)  $\times$  *G. Whitneyi*, different  $F^b$  plants. — *h*: 2678. 2 III + 4 II + 2 I. — *i*: 2696. 2 III + 3 II + 3 I. — *j*: 2660. heterochain + true III. — *k*: 2679. 4 II + hetero-III + homo-III. — *l*: 2679. 7 II. — *m*: 2680. 3 II + 2 I + 2 homo-III. — *n*: 2665. 7 II.

This was evident from the occurrence of multivalents or unequal II's or I's or an inversion bridge or a higher chromosome number than 14. CHITTENDEN (1928) has determined the somatic numbers in (*Whitneyi*  $\times$

*amoena*)  $\times$  *Whitneyi* and found  $2n$  to be 16, 15 or 14. These numbers were here found in the  $F^b$  plants. To describe meiosis in all these plants closely would be too burdensome; only a short summary will be given, and it must be remembered that the pairing was always variable.

One plant had 16 chromosomes; it was the  $F^b F^x f$  plant (2678). As many as three III's could be observed, one of them being a heterochain — in Fig. 1 *h* the small chromosome is free from the chain. Sometimes a chain-of-four occurred. Four plants had 15 chromosomes. One of them (2663) formed only one configuration-of-three, a heterochain. However, this plant must have had more than one *amoena* chromosome because one could sometimes observe a chain-of-four. Two of the 15-chromosome plants rather often formed two configurations-of-three, a true III and a heterochain with a small end-chromosome, the latter seeming to be smaller than in  $F_1$  (Fig. 1 *j* is from 2660). Plant 2696 also could form two chains, but the small chromosome seemed to be lacking here (Fig. 1 *i*); a chain-of-four rather often occurred. 2696 had much more often than  $F_1$  a bridge and an acentric fragment. The reason is probably that here there is a new inversion that arose in  $F_1$ .

14  $F^b$  plants were »disomics». Most of them could form at least two configurations-of-three, a heterochain with a small end-chromosome and a homochain or true III with triple chiasma (Fig. 1 *k*). This pairing, which may be written  $4\text{ II} + 2\text{ III}$ , was not so frequent. Other pairings were  $5\text{ II} + \text{I} + \text{III}$  or  $6\text{ II} + 2\text{ I}$ , univalents being of different size. The univalent could be small or large if only one chromosome was unpaired. A few plants of this type also had p.m.c.'s with 7 II, one II being heteromorphic (Fig. 1 *l*). Probably the small chromosome from the heterochain had paired with one of the chromosomes from the homochain; such a pairing could also occur in  $F_1$ . Some plants having two configurations-of-three were different; no clear case of a heterochain was observed, probably the small chromosome was not present. Fig. 1 *m* shows a pairing from such a plant, there is a true III and a chain of three chromosomes of about equal size.

Some disomics could show more than one unequal II. This is an indication that there are more *amoena* chromosomes than two. A higher number of *amoena* chromosomes must also be present in such disomics as could form a chain-of-four, an inversion bridge, or an usually high number of I's leading to a more or less frequent elimination at anaphase 1. The  $F^b$  plant having a *Whitneyi*-like mode of growth had often 7 II's, two of them heteromorphic (Fig. 1 *n*), but could sometimes

form a homochain. This plant could also show an inversion bridge; the small chromosome was lacking.

All  $F^b$  plants showed a chromosome number or a meiosis that indicated the presence of *amoena* chromosomes, and thus most probably the chromosome with  $F^b$ . The alleles  $W_1$ — $w_1$  were exchanged in  $F_1$  but an exchange of  $F$  alleles with loci in the same chromosome had not occurred. The high pollen sterility in  $F^b$  plants is no doubt due to the *amoena* chromosomes present, these causing a structural hybridity that must result in a high haplontic sterility. As mentioned, there seemed to be different degrees of pollen sterility, 70—80 % and more than 90 %. Meiosis in the more sterile plants showed a greater structural hybridity and thus the presence of more *amoena* chromosomes than plants having the lower degree of pollen sterility, i. e. plants with a chain-of-four, with several heteromorphic II's or several I's were of the more sterile type.

In most cases the number of *amoena* chromosomes must be lower than in  $F_1$  and meiosis is therefore less disturbed. Of more interest are such differences in the meiosis of backcross  $F^b$  and  $F_1$  as cannot be due to a different number of *amoena* chromosomes. Many  $F^b$  plants could form two configurations-of-three, one of them a heterochain. Such a heterochain could also be observed in  $F_1$ , but more rarely. Thus, in disomic backcross  $F^b$  p.m.c's with two configurations-of-three were more frequent than in  $F_1$ . The reason for this difference in pairing will be discussed in a following chapter. Metaphase 1 in  $F_1$  often showed bivalents formed of chromosomes of different size and form. An interchange in such a heteromorphic II could lead to new chromosomes having a changed size or form. Such new chromosomes were, however, not detected in the backcross plants. A study of chromosomes in root tips would perhaps have shown that new chromosomes may be formed. This is not improbable, since some backcross  $F^b$ 's seemed to show a reduction in size of the small chromosome in the heterochain. This is, however, a point that will be discussed later. A more certain change is perhaps a new inversion in one of the plants.

All  $F^b$  plants in the first backcross generation thus show sterility and the presence of *amoena* chromosomes. It is clear that the chromosome with  $F^b$  causes sterility. Four  $F^x$  plants were fertile and had a regular meiosis — 44  $F^x$  plants had, however, not been investigated. Now, HIORTH has made analogous crosses with the dominant *amoena* gene  $C^{sp}$  that causes a violet dusting on the leaves. The first backcross generation showed that  $C^{sp}$  segregates independent of  $F^b$ .  $F^b C^{sp}$  plants

always had, as expected, a high pollen sterility, but  $f C^{SP}$  also was sterile. Out of 7 plants investigated, 2 had a pollen sterility of about 30 %, 5 of about 80 %. Meiosis in one  $F^b C^{SP}$  and one  $f C^{SP}$  was studied. The former showed a rather small chromosome but was not investigated closely. As most common pairing  $f C^{SP}$  had 5 II + I + chain-of-three. The chain was formed of chromosomes of about equal size (Fig. 2 a), a heterochain was never observed, the small chromosome was not present. Of very rare occurrence was a chain-of-four (Fig. 2 b). The chain seemed very similar to the homochain in  $F_1$  and certain  $F^b$ :s in  $F_2$ . Thus, it seems that the  $C^{SP}$  chromosome from *amoena* also causes sterility when introduced into *Whitneyi* and is structurally different. Several  $F^x$ :s in the  $F^b$  cross must have had this  $C^{SP}$  chromosome and shown sterility.

( $F_1 \times G. Whitneyi$ ),  $F'_4$ . — The plants investigated belong to a different pedigree, the first backcross generation being sown in 1939. A number of  $F^b$  plants in this and the following backcross generation had been pollinated with pollen from *G. Whitneyi* plants that were  $F^x$  or  $f$ . In this way  $F'_4$  (sown in 1941) consisting of six families having 15  $F^b$  : 141 non- $F^b$  had arisen. All  $F^b$  plants in this as in the preceding generations were sterile. They had a long bent stem like *G. amoena*. One  $F^b$  (3054/795) had, however, a mode of growth more similar to that of *G. Whitneyi* and had a better fertility than other  $F^b$  plants in  $F'_4$ . Six plants were investigated.

Plant 3054 had 15 chromosomes and the pairing 6 II + heterochain (Fig. 2 c). The heterochain will be described later; it is composed of chromosomes that all are different. The small chromosome was sometimes unpaired, and the univalent formed may be divided or eliminated at anaphase 1 or 2. The absence of more I's and the fact that none of the II's showed any heteromorphy indicate that the six II's were formed by *Whitneyi* chromosomes. As will be shown below, the heterochain very probably has a *Whitneyi* chromosome in the centre, while the end-chromosomes are *amoena* chromosomes.

Four plants (3052, 3053, 3055, 3056) had a similar cytology. They had 14 chromosomes and could form two configurations-of-three. One of these configurations was the heterochain that was also formed by the trisomic plant. The small end-chromosome is smaller than the other chromosomes; this is also evident from somatic plates (Fig. 2 i). The second configuration-of-three is formed by two equal and one slightly smaller chromosome (Fig. 2 d). This configuration was very instabile; in most p.m.c's one of its chromosomes was univalent and hence this

configuration is rather difficult to study. Probably it is formed of one *amoena* and two *Whitneyi* chromosomes, but it seems that a triple chiasma may rarely be formed. The small chromosome from the heterochain is also rather often univalent, and the pairing  $6\text{ II} + 2\text{ I}$  is frequent, the I's being of a very unequal size (Fig. 2 *e*). Only

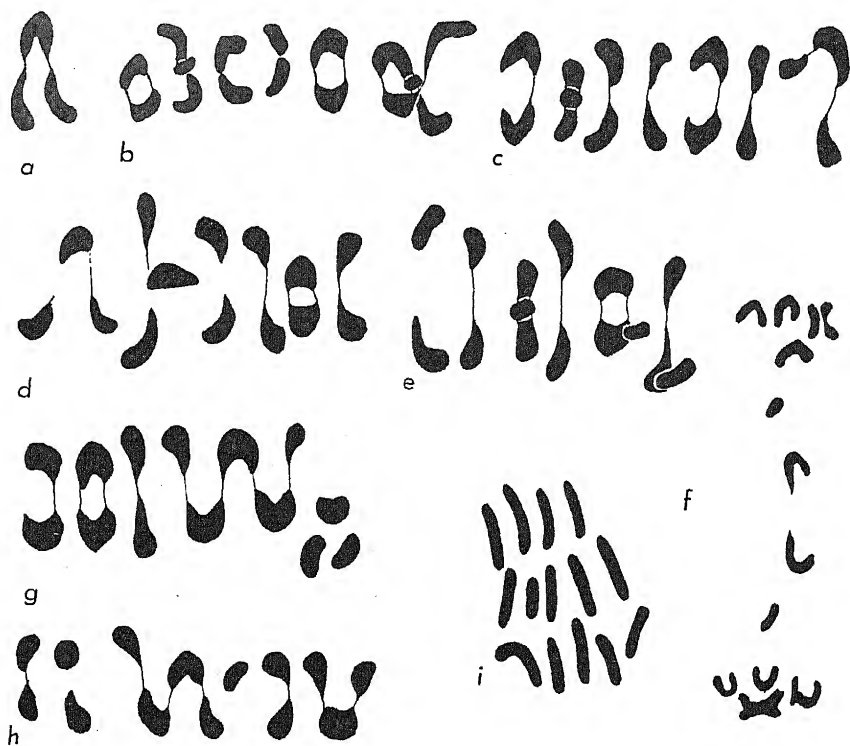


Fig. 2. *a-b*. (*G. amoena*  $\times$  *G. Whitneyi*)  $\times$  *G. Whitneyi*, *f* *C<sup>sp</sup>* plant. — *a*: homo-III. — *b*:  $5\text{ II} + \text{IV}$ . — *c-i*: (*G. amoena*  $\times$  *G. Whitneyi*)  $\times$  *G. Whitneyi*, *F<sup>b</sup>* plants from *F<sup>a</sup>*. — *c*:  $6\text{ II} + \text{heterochain}$ . — *d*:  $4\text{ II} + \text{III} + \text{heterochain}$ . — *e*:  $6\text{ II} + \text{large I} + \text{small I}$ . — *f*: division of I's at first anaphase. — *i*: somatic plate. — *g-h*: plant 3058. — *g*:  $\text{V} + 3\text{ II} + 3\text{ I}$ . — *h*:  $\text{V} + \text{III} + 3\text{ II}$ .

occasionally do more than 2 I's occur. Fig. 2 *f* shows the division of the two I's at anaphase 1.

One *F<sup>b</sup>* plant (3058) was a disomic having a different pairing. In many p.m.c.'s a chain-of-five could be observed with three large chromosomes in the centre and smaller end-chromosomes (Fig. 2 *g*). One end-chromosome in this chain was particularly small. As is shown in Fig. 2 *h*, this chain and a chain-of-three may occur in the same p.m.c.

The longer chain could be divided into a chain-of-four + I or a chain-of-three + II. This plant frequently had more than two I's and anaphase 1 was often rather disturbed.

The more sterile  $F^b$  plants were disomics and had more *amoena* chromosomes than 3054, this being shown by the formation of a second configuration-of-three. The great structural hybridity that thus is evidenced by the pairings observed explains this great sterility of the disomics. A greater number of *amoena* chromosomes also explains the *amoena*-like or intermediary mode of growth of the disomics, while 3054 was more similar to *Whitneyi*. It seems probable that the sterile  $F^b$  plants that have not been investigated were also disomics, most of them perhaps having the two configurations-of-three. The disomic 3058 with the chain-of-five came from a very sterile mother plant. As the offspring in this case was from an unisolated branch, pollen may have come from a *Whitneyi* with a chromosome ring. However, the assumption of the occurrence of a new translocation is needed to explain this unexpected pairing (cf. p. 253).

All II's in the disomic  $F^b$  were no doubt formed by *Whitneyi* chromosomes, this in contradistinction to the disomics with two configurations-of-three in  $F'_2$  that could show evidence of more *amoena* chromosomes. In no case was a heteromorphic II formed by chromosomes belonging to different chains observed, and the small chromosome in the heterochain here seemed to be smaller than the smallest chromosome in the  $F_1$  hybrid.

( $F_1 \times G. Whitneyi$ ),  $F'_5$  and  $F'_6$ . — In  $F'_4$  there seemed to be two different types of  $F^b$ . One plant (3054) represented what HIORTH has called the heterotrisomic  $F^b$  type having 6 II + heterochain, the others were of what HIORTH called the disomic sterile  $F^b$  type having the heterochain and a second configuration-of-three. These types were also found in later backcross generations, but while in  $F'_4$  most  $F^b$  plants were of the disomic sterile type, only one of the  $F^b$ 's from later generations that had been fixed belonged to this type. The heterotrisomics are more fertile and are similar to *G. Whitneyi* in mode of growth. HIORTH found in 54 plants of the latter type 25—81 % poor pollen with a mean of 47 % (all these plants had not been investigated cytologically). Plants of the sterile type had more than 80 % poor pollen and always had a much lower seed fertility than the heterotrisomics. Thus, six sterile  $F'_4$  plants after backcrossing gave 21  $F^b$  : 92 non- $F^b$ , while the one heterotrisomic gave 20  $F^b$  : 70 non- $F^b$ .

From the offspring last mentioned (the family 882—1942) that had

3054 as mother three  $F^b$  plants were studied. In the next backcross generation  $F^b$ , the segregation had been in all 26  $F^b F^x$ : 376  $F^x F^x$ . A few  $F^b$  individuals from this generation (808—1943) were also investigated. All plants were heterotrisomics, and meiosis could be studied more thoroughly than in 3054. The small end-chromosome in the

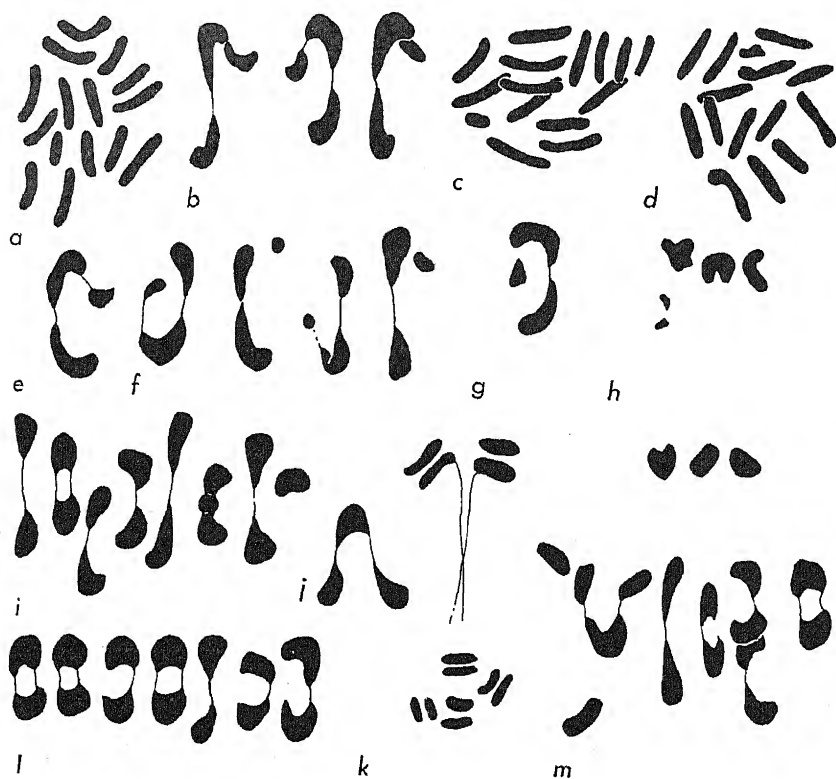


Fig. 3. (*G. amoena*  $\times$  *G. Whitneyi*)  $\times$  *G. Whitneyi*.  $F^b_5$  and  $F^b_6$ . — a: heterotrisomic  $F^b$ , somatic plate. — b: heterotrisomic  $F^b_1$ , heterochains. — c:  $F^x$  plant 1138/886, somatic plate. — d:  $F^b$  plant 1131/886, somatic plate. — e: 1138/886, heterochain. — f—g:  $F^b$  from 846—1943, heterochains. — h: 845—1942, elimination of fragment chromosome at first anaphase. — i—k: the  $F^b$  plant 1171/889. — i: III + 6 II. — j: the configuration-of-three. — k: bridge formation at first anaphase. — l: fertile  $F^b$ , 7 II. — m: the  $F^b$  plant 2669/817—1943, III + 5 II + 2 I.

heterochain measured 7 mm. at a magnification of  $\times 5000$ , the larger end-chromosome 12 mm. The former had a submedian centromere, the latter a subterminal. The chromosome in the centre was heterobrachial, the short arm formed chiasma with the small chromosome. The centro-

meres of the end-chromosomes are orientated towards one pole, the chromosome in the centre towards the opposite pole. This must cause a rather constant distribution of the chain at anaphase 1, the end-chromosomes going to the same pole. Exceptions from this distribution may occur. One cause of such exceptions is a more neutral orientation of the small chromosome, its centromere not being directed towards any definite pole (cf. Fig. 3 *g*), another cause is univalence of the small chromosome. In one slide as many as  $\frac{1}{3}$  of the p.m.c's had a small I, but other slides showed a lower frequency, and an elimination of the small chromosome occurred in less than 20 % of the p.m.c's.

The appearance of the chain was somewhat variable. The small chromosome could be connected by a thread but perhaps more often the connection was closer (Fig. 3 *b*). The third mode of connection, through an interstitial chiasma, was never observed, nor was such a chiasma formed by the larger end-chromosome. The II's are as a rule open, only 0—2 ring-II's were observed in the p.m.c. A somatic plate is shown in Fig. 3 *a*. It is very probable that all  $F^b$ 's in these families were heterotrisomics.

An interesting  $F^b$  family was 886—1942 from a cross  $F^b F^x \times ff$ . The segregation was 5  $F^b f$  : 14  $F^x f$ . The mother plant, 3051/795—1941, had unfortunately not been investigated. HIRTH mentions that it belongs to the sterile, disomic type. All  $F^b$  and 5  $F^x$  plants were investigated. The  $F^b$  plants were heterotrisomics, and the small chromosome had a reduced size (Fig. 3 *d*). While, as a rule, it has more than half the length of the larger end-chromosome, it is here less than half of this chromosome, at a magnification of 5000 measuring 4.5—5 mm. at first metaphase. The orientation of the chromosomes of the heterochain is the usual, at anaphase 1 the end-chromosomes pass to the same pole. Here, more often the small chromosome is at a distance from and connected by a thread to the chromosome in the centre.

Four  $F^x$  plants had, as expected, 7 II and had no doubt 14 *Whitneyi* chromosomes. One  $F^x$  (plant 1138) was, however, heterotrisomic; it had the small chromosome (Fig. 3 *c*). The heterochain was somewhat different from the chain of the  $F^b$  sisters. It was the larger end-chromosome that was different, this having the same size and form as the chromosome in the centre (Fig. 3 *e*). The chain thus has two *Whitneyi* chromosomes, and 1138 as normal  $F^x$  plants 14 such chromosomes. The number of ring II's were rather high, 3—4 in most p.m.c's. Plant 1138 had a pollen sterility of 27 %, while four normal  $F^x$  sisters had only 3—6 %. After selfing, 1138 has given 152  $F^x W_1$  : 21  $F^x w_1$  : 18  $f W_1$  :

29  $f w_1$ . This segregation is quite normal and shows that the small chromosome has no influence on the segregation (HIORTH, l. c.). The cause of the pollen sterility is unknown. The small chromosome was often univalent.

The  $F^b$  plant 1131/886 had in a cross  $F^b f \times ff$  unexpectedly given the segregation 24  $F^b f$  : 35  $ff$  (= the family 846—1943). Selfing of 1131 also gave an unexpectedly high number of  $F^b$  plants. Of 846—1943 one  $f$  and seven  $F^b$  plants were investigated. The  $f$  had 7 II, all  $F^b$ :s were heterotrisomics with a very small chromosome (Fig. 3  $f$ ), similar to that in the mother plant. A division of the fragment chromosome is shown in Fig. 3  $h$ . The  $F^b$  plants in 846 seemed to be very sterile, five plants pollinated with  $f$  pollen giving offspring of only 103 plants, 6  $F^b f$  : 97  $ff$ . This is again unexpected, the number of  $F^b$  now being low, perhaps owing to the sterility (HIORTH). The unusual segregation of 1131 could not be due to the reduced size of the small chromosome because sister plants have given the ordinary heterotrisomic segregation.

The small chromosome of the heterochain may also be larger than in 882—1942; this was possibly the case in the only  $F^b$  plant in 814—1943. The mother plant had not been investigated but was of the sterile type (HIORTH).

A cross of the type  $F^b F^x \times ff$  had given 1  $F^b F^x f$  : 1  $F^b f$  : 19  $F^x f$  (the family 889—1942). The mother plant was 3056 from  $F'_4$ , a disomic having two configurations-of-three. Plant  $F^b f$  was a heterotrisomic with the usual heterochain. Plant  $F^b F^x f$  (1171/889) also had 6 II and a configuration-of-three, but it was not the heterochain, being formed of two chromosomes of equal size and one that was slightly smaller (Fig. 3  $i$  and  $j$ ). A triple chiasma was sometimes observed. The pairing could also be 7 II + I or 6 II + 3 I. Anaphase 1 was very disturbed, there being frequent laggings and eliminations. Bridges were observed, but no acentric fragments. Fig. 3  $k$  shows a bridge uniting two chromosomes from the III going to the same pole. It is clear that 1171 lacks the small chromosome, and the constitution  $F^b F^x f$  shows that a non-disjunction had probably occurred in the mother plant. Thus, the new configuration was probably composed of two *Whitneyi* chromosomes and the larger end-chromosome from the heterochain, because in the mother plant the chromosome in the centre and the larger end-chromosome had gone to the same pole. 1171 is the homotrisomic  $F^b$  type of HIORTH.

889—1942 showed that a disomic  $F^b$  had produced a heterotrisomic  $F^b$ . In a similar cross with a cytologically investigated disomic  $F^b$  from

$F''_4$  only one  $F^b$  was examined and this plant had 4 II + 2 configurations-of-three as the mother plant (888—1942).

In  $F''_5$  appeared the first  $F^b$  plant that was fully fertile: the fertile  $F^b$  type. This plant had a pollen sterility of only 2 %, after selfing it gave 67  $F^b F^b$  : 213  $F^b F^x$  : 115  $F^x F^x$ , that is 71 %  $F^b$ , 17 %  $F^b F^b$ , while the heterotrisomic segregates 12.2 %  $F^b$  and 0.21 %  $F^b F^b$ . The fertile plant (1107/883—1942) was not investigated, however, two  $F^b F^b$  and two  $F^x F^x$  individuals from the progeny showed 7 II (Fig. 3 l) and a regular meiosis; an  $F^b$  plant from Bremen  $\times$  1107 also had 7 II. There was no trace of heteromorphy in any II, and the fertile  $F^b$  no doubt had only *Whitneyi* chromosomes.

HIORTH has obtained the fertile  $F^b$  type 12 different times; it has appeared in the offspring from heterotrisomic  $F^b$ , disomic sterile  $F^b$  or the homotrisomic 1171. Plants belonging to eight of these lines have been investigated, they all had 7 II, in certain plants a weak asynesis sometimes causing I's was manifested. The segregation of the fertile type is normal. In several cases HIORTH, however, found too few  $F^b F^b$  plants. Crossing-over in  $F^b$ — $W_1$  was normal (26 %). Some plants had a certain amount of pollen sterility (in one case 43 %).

Finally, the family 817—1943, the cross  $ff \times$  1106/883, may be mentioned. The male parent 1106 had not been investigated; it was a sterile  $F^b$ . The  $F^b$  plants in 817 showed a very different sterility. One plant had a pollen sterility of 7 % and had 7 II. One plant having a pollen sterility of 73 % was a trisomic with a heterochain. Elimination was rather frequent at anaphase 1, and bridges were seen. A third plant (2269/817) with a sterility of 38 % was a trisomic that formed a chain of chromosomes of rather equal size. One of these chromosomes was rather often univalent and could be eliminated at anaphase 1. In a letter HIORTH has communicated the results of crossings with this plant. Selfing of 2669 has given 13  $F^b W_1$  : 2  $F^b w_1$  : 10  $f W_1$  : 26  $f w_1$ , and  $f w_1 \times$  2669/817 gave 56  $F^b W_1$  : 5  $F^b w_1$  : 19  $f W_1$  : 176  $f w_1$ . Several  $F^b$  plants in the offspring showed a similarity to *G. amoena*. HIORTH finds these results indicate that 2669 is a  $F^b$  of the fertile type. The configuration-of-three was, as a rule, a chain having a rather large chromosome with a submedian centromere in the centre, the end-chromosomes being slightly smaller. These latter are perhaps *amoena* chromosomes (Fig. 3 k). The same configuration must have been present in 1106/883.

*Conclusions.* — With the production of a fertile  $F^b$  type the problem of transferring  $F^b$  to *G. Whitneyi* has been solved. To begin with, how-

ever, the interesting heterotrisomic  $F^b$  type that first appeared in  $F'_4$  will be discussed. The heterotrisomic cannot have more than two *amoena* chromosomes, the end-chromosomes of the heterochain. As a rule, it forms two kinds of fertile gones,  $F^x$  or *Whitneyi* gones having 7 chromosomes including the chromosome in the centre of the heterochain, and  $F^b$  gones having 8 chromosomes including the end-chromosomes of the chain. Elimination of the small chromosome must rather often cause the formation of gones having 7 chromosomes including the large end-chromosomes but lacking the small. The results show that such gones must be sterile. Also a gone with the small chromosome and having only 7 chromosomes is sterile, for no plant with  $13 +$  the small chromosome has been found. Thus the two end-chromosomes in a way complement one another to substitute the *Whitneyi* chromosome of the heterochain and give a fertile gone. As most sterile  $F^b$  plants had the small chromosome, one might be inclined to think that the gene  $F^b$  is in the small chromosome. However, this does not seem to be the case,  $F^x$  having the small chromosome has been found, and also  $F^b$  (sterile) lacking the small chromosome. A view more in harmony with the observations is that  $F^b$  has its locus in the larger end-chromosome — the presence of the small chromosome in most  $F^b$  plants is a result of the distribution of the chromosomes of the heterochain and of the fact that it supplements the  $F^b$  chromosome to give a fertile gamete. HIORTH designated the small chromosome 2.3', the larger end-chromosome 1.6', the *Whitneyi* chromosome being 1.2 (Fig. 4). The heterochain, thus, is 6'.1—1.2—2.3', the ends marked ' are according to HIORTH not able to form a chiasma with corresponding *Whitneyi* segments. The sterility of  $F^b$  gones with 7 chromosomes is due to the absence of segment 2. The chromosomes of *G. Whitneyi* have been numbered 1.2, 3.4, 5.6, and so on. One could never observe the heterochain in connection with any of the 6 *Whitneyi*-II's. This must mean that the free ends of the two *amoena* chromosomes cannot form a chiasma with »homologous» *Whitneyi* ends 3 and 6.

The assumption that the  $F$  alleles have not loci in the chain but in a bivalent, and that the  $F^b$  chromosome of this bivalent must be supplemented by the small chromosome, is in conflict with many facts. Then the III of  $F^b F^x f$  (plant 1171/889) would be composed of three identical chromosomes, which, however, is not the case.

The small chromosome is more or less frequently unpaired. In view of its small size and of the fact that it comes from a foreign genome this is not surprising. In  $F_1$  the corresponding chromosome

was still more often univalent, or it could form a II with a chromosome from a different configuration-of-three; a heterochain was rare in  $F_1$ . The larger *amoena* chromosome was never unpaired in the heterotrisomic. But also in  $F_1$  it was probably rather rarely univalent: in  $F_1$  a regular crossing-over of the genes  $W'_1$  and  $w_1$  linked to  $F$  alleles is as high as in intraspecific crosses with races of *Whitneyi*. This indicates a regular pairing between these chromosomes in the  $F_1$  hybrid;  $W'_1-w_1$  must have loci near the ends of the chromosome arms that form the chiasma, that is, near the end of the longer arms 1. An interchange must here be frequent, but it would by no means be surprising if the pairing of these arms was still more frequent in later generations, these interchanges having made the long arm of the  $F^b$  chromosome more similar to the homologous *Whitneyi* arm. Frequent interchanges do not, however, occur in the part of the chromosomes containing loci of the  $F$  alleles. Exchanges between the larger end-chromosome and the *Whitneyi* chromosome of the chain perhaps also explain the absence of an interstitial chiasma in later generations.

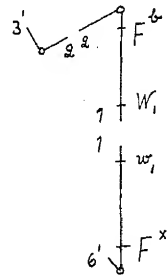


Fig. 4. Diagram of the heterochain.

The pollen sterility of heterotrisomic  $F^b$  must be caused by the elimination of the small chromosome. However, many trisomics had a much higher pollen sterility than one would expect after the elimination observed. HIRTH assumes that secondary causes often increase this primary elimination sterility. The rather low number of  $F^b$  plants in the offspring is explained by the fact that  $F^b$  gones have  $n + 1$  chromosomes, two of them coming from a foreign species. When used as male parent, the heterotrisomic usually gives only slightly more than 1 %  $F^b$ ; the transmission of  $F^b$  through the egg-cells is better, selfing giving 12.2 %.

At least in two cases an unusual distribution of chain chromosomes has resulted in aberrant plants, once an  $F^x$  having 14 *Whitneyi* chromosomes and the small chromosome, and once an  $F^b$  having 14 *Whitneyi* chromosomes and the  $F^b$  chromosome (plant 1171/889; p. 246). In the former case the small chromosome had passed to the same pole as the chromosome in the centre of the chain, in the latter case the larger end-chromosome had followed the chromosome in the centre. 1171/889 was  $F^b F^x f$ , and it seemed unable to transmit the  $F^b$  chromosome through the pollen. In crosses where 1171 is the male parent it has given 0  $F^b F^x$  : 3  $F^b$  : 222  $F^x$  : 177  $f$ . One  $F^b$  was investigated; it

had 7 II and thus belonged to the fertile type lacking the  $F^b$  chromosome 1.6'. Probably the two  $F^b$ :s not investigated also had 7 II. Selfing 1171 has given 8  $F^b$  : 110 non- $F^b$ ; an  $F^b$  investigated had 7 II. It seems, however, probable that egg-cells having 7 *Whitneyi*- + the  $F^b$ -chromosome may function, though more rarely than egg-cells with 6 *Whitneyi*- + the  $F^b$  + the small chromosome in heterotrisomics.

The fertile  $F^b$  plants had 7 II. No doubt the fertile type has only *Whitneyi* chromosomes, crossing-over in the heterochain having transferred the gene  $F^b$  to the chromosome 1.2. In all plants with a chain having the  $F^b$  chromosome 1.6' crossing-over could occur, and fertile  $F^b$  has been produced by disomic sterile  $F^b$ , heterotrisomic  $F^b$  and the »homotrisomic» 1171, as one could expect. Relatively often it has appeared in crosses where heterotrisomic  $F^b$  was the male parent. HIORTH attributes this to the fact that the cross-over pollen grains must be more vital than the usual  $F^b$  grains with 8 chromosomes. However, crossing-over must be very rare.  $W_1$  of the same linkage group had, as mentioned, more than 20 % crossing-over and this difference must be due to a different position of the genes,  $F$  alleles being nearer the centromere,  $W_1$  near the end of the long arm of the chromosomes (Fig. 4). It is improbable that  $F^b$  has its locus in the short arm, no chiasma being observed there and these arms being of different size in 1.2 and 1.6'. HIORTH has shown that in the fertile type  $F^b$  must be allelic to or show absolute linkage with  $F^x$  of *Whitneyi*, as crosses  $f \times F^b F^x$  have given 356  $F^b f$  : 447  $F^x f$ . In most crosses with the fertile type too few  $F^b F^b$  plants appear; the transmission of  $F^b$  is better through the pollen than through the egg-cells.

On p. 247 reference is made to plant 2669/817, which segregates like the fertile type but has *amoena* chromosomes. There was probably a cross-over chromosome in the pedigree, as also seems to be evident from the repeated production of fertile  $F^b$  plants. This chromosome was probably in the  $F'_4$  plant 3048—1941 (not investigated),  $3048 \times F^x$  had produced 3  $F^b$  plants, one fertile (7 II), two very sterile. One of the sterile plants was mother of the heterotrisomic in 814—1943. The second sterile plant (1106) produced several fertile  $F^b$ :s. In the offspring after selfing 1106 two  $F^b$ :s with 7 II were found, and in  $f \times 1106$  one heterotrisomic  $F^b$  and two having the gene  $F^b$  in a bivalent. These facts are perhaps explicable on the assumption that 3048—1941 had a heterochain with a cross-over chromosome in the centre and a second configuration, viz. the one shown in 2669/817.

There is a disomic  $F^b$  type which is very sterile. Heterotrisomics

could also show a rather high pollen sterility but always had a much lower seed sterility. The disomics had the heterochain, but a second configuration-of-three showed the presence of more *amoena* chromosomes. This configuration is probably composed of 2 *Whitneyi* chromosomes and 1 *amoena* chromosome. It has been shown that in the offspring of  $F^b$  disomics of this cytological type there may be  $F^b$  plants with the same pairing, heterotrisomics, »homotrisomics» and fertile  $F^b$ :s. If the end-chromosomes of the heterochain and the two *Whitneyi* chromosomes in the second configuration-of-three go to the same pole, gones are formed giving heterotrisomic  $F^b$  in the backcross. If it is the *amoena* chromosome of the second configuration that follows the end-chromosomes of the heterochain, then a plant like the mother is formed. The *amoena* chromosomes are no doubt the cause that  $F^b$  gones function much more rarely than  $F^x$  gones having 7 *Whitneyi* chromosomes. In later generations disomic sterile  $F^b$  plants seem to be rare, and such plants are of course never formed by heterotrisomic  $F^b$ .

HIORTH has preliminarily numbered the chromosome ends in the second configuration as 3.4—4.5—5.6, the chromosome 4.5 being from *amoena*. This configuration could not be studied so well as the heterochain; it is more instabile and may show a triple chiasma, indicating a more complicate structure than these numbers show. A number of the plants that have been used in crosses and that HIORTH considers to be of the disomic, sterile type have not been investigated. All disomic sterile  $F^b$  plants do not show this pairing; some perhaps had more than three *amoena* chromosomes. The second configuration is different from the homochain in  $F_1$  and  $f C^{sp}$ .

The small chromosome may have a different size. It cannot be doubted that in  $F'_4—F'_6$  it is smaller in most families than in  $F_1$ . In a pedigree coming from plant 3051/795—1941 it was particularly small. Here the mother plant must have a chromosome of diminished size. The cause of this variation in size of the small chromosome is perhaps an exchange with the chromosome in the centre, this exchange being unequal. Exchanges between these two chromosomes may also explain the fact that a chain with a small chromosome that is rather rarely formed in  $F_1$  is more often observed in certain plants of  $F'_2$  and still more often in later backcross generations. A second cause of the rareness of heterochains in  $F_1$  and certain  $F'_2$  plants is that the small chromosome sometimes forms a bivalent with a larger chromosome from a homochain or III. Such a behaviour of the small chromosome was not observed in  $F'_4—F'_6$ , the second configuration-of-three of these di-

somics is, as it seems, not identical with the homochain or III so often observed in  $F_1$  or  $F'_2$  and in  $f^{C^{sp}}$ . In  $F_1$  and  $F'_2$  one would sometimes expect a chain-of-six; such a configuration has, however, not been observed.

Exchanges between *amoena* and *Whitneyi* chromosomes in the heterochain might thus give an explanation not only of the better pairing but also of the size reduction of the small chromosome. But unequal exchanges should also give rise to a »small» chromosome increased in size. Whether such an increase has actually occurred is not clear.

Two *amoena* chromosomes have caused pollen sterility in backcrosses to *Whitneyi*, the  $F^b$  chromosome and the  $C^{sp}$  chromosome. The former is a member of a heterochain and thus structurally different from homologous *Whitneyi* chromosomes, the latter is also structurally different, being very probably a member of a chain-of-three or -four.

DOBZHANSKY speaks of two different kinds of hybrid sterility, genic and chromosomal. At first the sterility of the  $F^b$  plants in the crosses seemed to be a genic sterility; it is, however, a case of chromosomal sterility, crossing-over of the gene to a *Whitneyi* chromosome having removed the cause of sterility.

Probably still more *amoena* chromosomes produce sterility when introduced into the *Whitneyi* genome. Other *amoena* chromosomes may be structurally similar to and regularly pair with the corresponding *Whitneyi* chromosome. This is probable also from the fact that the number of I's in  $F_1$  is always low, and some II's seem to be strictly homomorphic. Such chromosomes may easily replace homologous *Whitneyi* chromosomes. But clearly the important morphological species differences in *amoena*—*Whitneyi* have no loci in such easily interchanged chromosomes. It is true that many structural differences may occur in intraspecific races or hybrids in *G. Whitneyi*, but the rather constant ring formation and a regular assortment of ring chromosomes here results in fertile gones. In the interspecific crosses larger rings are not formed, certain »homologous» segments often or always being unpaired and the result being a more independent distribution of the structurally different chromosomes leading to haplontic sterility. It has been mentioned that HIORTH considers the pairing  $2\text{ II} + \text{ring-of-four} + \text{ring-of-six}$  as that which would be shown by *amoena*  $\times$  *Whitneyi* if all homologous ends were united at metaphase 1. The ring-of-four should contain the  $C^{sp}$  chromosome, the ring-of-six the chromosomes of the two configurations-of-three in disomic sterile  $F^b$ .

In two cases chromosome changes not involving the small chromosome had manifested themselves. One was a new inversion in  $F'_2$  and the second was a reciprocal translocation involving the 6' end in  $F'_4$ . Plant 3058, mentioned on p. 242, had a chain-of-five with 2 small end-chromosomes. One end-chromosome was probably 2.3' and therefore the  $F^b$  chromosome should be the chromosome in the centre of the chain. This chromosome is (a) larger than the normal  $F^b$  chromosome 1.6' and has a submedian centromere, (b) forms a chiasma in both its arms. These facts speak in favour of the view that the chromosome 1.6' has made a reciprocal exchange involving the end 6' that now should belong to the second end-chromosome.

### SOME NEW CASES OF SPONTANEOUS CHROMOSOME CHANGES IN GODETIA.

That the chromosomes of *Godetia* must be very liable to structural changes is already indicated by the common occurrence of rings or chains at metaphase 1. More rare than reciprocal translocations are inversions. Fragmentations or deletions where a chromosome shows a reduced size while a corresponding increase in size in other chromosomes could not be observed, have been found after X-raying. HIORTH produced aberrant plants of *G. Whitneyi* by pollination with X-rayed pollen. Three aberrants had a diminished chromosome (HÅKANSSON, 1940). One was a trisomic with a very small chromosome that sometimes formed a hetero-III with two chromosomes of normal size. Two were disomics that often formed a hetero-II; in one of them the fragmented chromosome was very small. A spontaneous chromosome fragmentation has been found in the allotrisomic *Rn* (HÅKANSSON, 1941), and some new cases of this type will be described here because they were accompanied by certain genetical peculiarities.

Backcrossings of the type (*Godetia deflexa*  $\times$  *G. Whitneyi*)  $\times$  *G. Whitneyi* have produced several different allotrisomic types having 14 *Whitneyi* chromosomes and 1 *deflexa* chromosome (HIORTH, 1946 a). At first metaphase they have the pairing 7 II + I (HÅKANSSON, 1941). They are similar to *G. Whitneyi* but show certain morphological characters from *deflexa*. Each of them must have a different *deflexa* chromosome and is called after the most striking *deflexa* character shown.

The allotrisomics described by HIORTH are: (1) *Kl* (= kleinblütig) has small flowers, a very high pollen sterility (98 %), in *Kl* ♀  $\times$  normal ♂ there is an excess of *Kl* plants (62 instead of 50 %), (2) *Rn* (= rotnervig), the middle rib of the leaves

has a red colour;  $Rn \text{♀} \times \text{normal } \text{♂}$  gives too few  $Rn$  plants (11,8 %); in normal  $\text{♀} \times Rn \text{♂}$  only 3,5 %  $Rn$  appear. X-raying the pollen of  $Rn$  has produced a translocation called  $Rn^t$ , a segment of the *deflexa* chromosome had been translocated to a *Whitneyi* chromosome.  $Rn^t$  has only 14 chromosomes and forms 7 II, one of them a hetero-II (HÅKANSSON, 1943). In  $Rn^t \text{♀} \times \text{normal } \text{♂}$  a much higher number of plants appear with red ribs on the leaves (39,9 %), in normal  $\text{♀} \times Rn^t \text{♂}$  10,9 %. Homozygous  $Rn$  is unknown.  $Rn^t Rn^t$  is, however, vital. It has no hetero-II (HÅKANSSON, 1944) and is a rather weak type, having smaller flowers and leaves than and being only about half the height of heterozygous  $Rn^t$ . (3)  $Pu$  (= punktiert) has violet points on the petals. In  $Pu \text{♀} \times \text{normal } \text{♂}$  only 6,9 % are  $Pu$ , and in crosses of the type normal  $\text{♀} \times Pu \text{♂}$  there is only 0,13 %  $Pu$ . Spare pollination, which in the cross normal  $\text{♀} \times Rn \text{♂}$  considerably increases the frequency of  $Rn$ , is of no influence here. HIORTH suggests as an explanation that the growth of the pollen tube is entirely dependent on the tube nucleus, that  $Pu$  is lethal to the tube, and that the few  $Pu$  plants formed are the result of an elimination of the  $Pu$  chromosome at the first pollen mitosis, with the result that a  $Pu$  chromosome is present in the generative nucleus but not in the tube nucleus.

A plant with a diminished chromosome was detected in  $F_1$  from a cross between  $Kl$  and a recessive *Whitneyi* strain  $gg$ .  $gg$  plants have a green-light red stem, while  $G$  causes a red stem colour. In  $Kl GG \times gg$  one of the disomic  $F_1$  plants had, however, a green stem showing the recessive character (HIORTH, 1946 a). This plant was a variant having reduced size and buds, more hairy than usual. The cytological investigation showed that this plant had 14 chromosomes, one of them being very small. In Fig. 5 *e* is a plate from a young petal; the small chromosome is about  $\frac{1}{4}$  of a normal chromosome. Figs. 5 *a*—*c* show the chromosome pairing at metaphase 1. In *b* the fragment chromosome is paired with a normal chromosome. However, in most p.m.c.'s the fragment chromosome is unpaired; in Fig. 5 *a* there is 6 II + I + fr., and in *c* chain-of-three + 5 II + fr. Fig. 5 *d* shows late anaphase 1; the fragment chromosome is belated. It may be added that in a few loculi the fr. chromosome seemed to be absent, indicating that it had been eliminated at mitosis.

It is clear that this fragment chromosome has been formed by a maternal  $G$  chromosome which has lost most of its substance, including the gene  $G$ .

A second case of a spontaneous chromosome fragmentation is in  $Rn^t Rn^t$ . HIORTH has crossed this form and the standard race Bremen. In  $Rn^t Rn^t \text{♀} \times \text{Bremen } \text{♂}$  all  $F_1$  plants (1000) were  $Rn^t$ , as expected. Three of them were investigated and the heteromorphic II that characterizes heterozygous  $Rn^t$  could be observed in all. Reciprocal crosses, Bremen  $\text{♀} \times Rn^t Rn^t \text{♂}$ , gave progenies of 2003 plants; however, no fewer than 54 had a green rib on the leaves, thus unexpectedly showing

the recessive character. I have investigated ten of these aberrant plants. Two of them were haploids (the haploidy was suspected by HIORTH); they have arisen by parthenogenesis. Eight of them were disomics with 7 normal II's; no hetero-II could be observed. It is clear that the translocated *deflexa* segment is not present in the aberrant  $F_1$  plants with green ribs on the leaves. In the homozygote  $Rn^t Rn^t$  the *Whitneyi* chromosome carrying the *deflexa* segment is liable to changes leading to the loss of this segment.

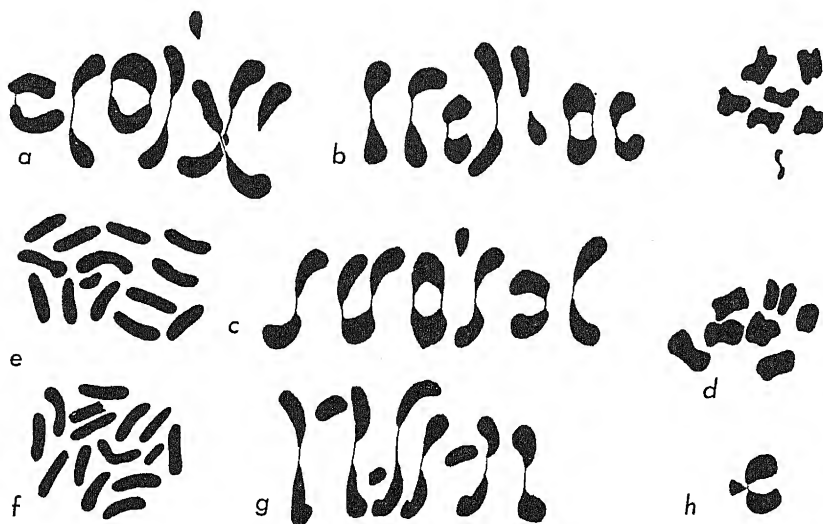


Fig. 5. *a—c*. Allotrisomic  $Kl GG \times gg F_1$ , *gg* plant. — *a*: 6 II + I + fr. — *b*: 7 II. — *c*: III + 5 II + fr. — *d*: elimination of fr. chromosome at first anaphase. — *e*: somatic plate. — *f—h*. *G. Whitneyi*, trisomic  $Cp^a$ , plant with  $Cp^a$  fragment chromosome. — *f*: somatic plate. — *g*: 6 II + 2 I + fr. — *h*: hetero-III.

Two of the green-ribbed plants were semisterile. HIORTH suggests that this sterility can be explained by the assumption that a minor part of the *Whitneyi* chromosome was lost at the same time as the *deflexa* segment. In several plants a number of the p.m.c.'s had 6 II + 2 I. This was also found in 15 % of the p.m.c.'s of  $Rn^t Rn^t$ . The cause of this asyndesis is not clear. A strange effect of the rather frequent loss of the *deflexa* segment is that homozygous  $Rn^t$  is not quite constant. In the progeny of  $Rn^t Rn^t$  HIORTH detected some  $Rn^t$  heterozygotes, that is, plants distinctly larger than the homozygotes. Selfing of these larger plants resulted in an offspring segregating in plants with red and with green ribs, and thus the forming of  $Rn^t$  heterozygotes from  $Rn^t$  homozygotes is proved.

It is interesting that the foreign segment seems to be rather loosely attached to the *Whitneyi* chromosome. Probably the loss of the segment occurs at meiosis in the p.m.c., but this has not actually been observed in the slides. There is so far no evidence that the segment also may be lost in the e.m.c. HIORTH, however, stresses the fact that pollen tubes with  $Rn^t$  do not function so well as normal tubes, backcrosses with the heterozygote as male parent giving only 10.9 %  $Rn^t$  instead of, as expected, 50 %. Loss of the segment may thus give rise to pollen grains functioning far better than the  $Rn^t$  grains, and relatively many plants with a green midrib are the result. That no such plants appear in crosses where  $Rn^t Rn^t$  is the female parent could largely be explained as a result of the absence of such a concurrence in the female sex.

A third case of fragmentation of a genetically marked chromosome is known from the crosses of HIORTH with *Whitneyi* plants having the dominant gene  $C^{pa}$  (HIORTH, 1946 c).  $C^{pa}$  causes certain patterns on the cotyledons, their base having a reddish violet colour and a red arrow appearing higher on the cotyledon. Very young leaf buds are also reddish. Most  $C^{pa}$  are monosomic complex heterozygotes *a.g* having the pairing 4 II + I + chain-of-four, *a* being the standard complex of *G. Whitneyi*, *g* being a complex with only 6 chromosomes.  $C^{pa}$  has its locus in a *g* chromosome of the chain, and the frequent elimination of the I, which is an *a* chromosome, results in a great excess of  $C^{pa}$  in crosses  $C^{pa} \times c.c.$  There is, however, a disomic  $C^{pa}$  with the pairing 5 II + chain-of-four that showed a normal segregation of  $C^{pa}$  in female backcrosses. Now, a third  $C^{pa}$  type has been found which in such crosses gives only few  $C^{pa}$  plants. This type is trisomic with the pairing 6 II + III or 7 II + I, and no doubt is *a.a* + the  $C^{pa}$  chromosome. It must have arisen through a non-disjunction in a monosomic, the  $C^{pa}$  chromosome having followed the *a* chromosomes of the chain. Four  $C^{pa}$  plants in the offspring of a cross with this new type were investigated. Three plants showed an extra chromosome of normal size; in one, however, there was only a fragment chromosome about  $\frac{1}{4}$  the size of the normal chromosome (Fig. 5 f). In most p.m.c.'s the fr. chromosome was unpaired (Fig. 5 g), though it could form a hetero-III with two normal chromosomes (Fig. 5 h), but no hetero-II was observed. These facts make it probable that the small chromosome is a fragment of the  $C^{pa}$  chromosome still containing the gene  $C^{pa}$ . Elimination of the small chromosome was not infrequent at anaphase 1.

HIORTH has had progenies of the plants that had been studied

cytologically. The fragment plant showed a better pollen and seed fertility than the plants with a normal  $C^{pa}$  chromosome, although the segregation was very similar to that of the latter plants. This was perhaps unexpected: »... man sollte annehmen, dass Gameten mit Fragment besser funktionstüchtig sind, als solche mit einem ganzen überzähligen Chromosom. Dies dürfte indessen durch eine höhere Elimination des Fragmentes ausgeglichen werden» (HIORTH). The family originating from the fragment plant could only be studied by HIORTH in the seed bed.  $C^{pa}$  plants here showed a striking peculiarity. The cotyledon pattern was more marked than usual, and all seedlings had one or more green sectors surrounded by reddish-violet tissue. One seedling had a violet and a green cotyledon. In the three sister families having a normal  $C^{pa}$  chromosome no seedling showed any green sector. These facts seem to prove that it was the  $C^{pa}$  chromosome that had been fragmented. The green sectors must have arisen through somatic elimination of  $C^{pa}$ . The fragment chromosome must be rather frequently eliminated at mitosis, while a normal  $C^{pa}$  chromosome is not eliminated. A study of the process of elimination was not attempted because no fixation of root tips could be procured. Thus, the cause of this different behaviour of the changed  $C^{pa}$  chromosome cannot be discussed.

### CONCLUSIONS.

Several cases of spontaneous chromosome changes have been found during cytological investigations of the  $F^b$  plants in the successive back-cross generations, others have been detected after an investigation of material that had shown an aberrant genetical behaviour. In the species cross a change had once been brought about through a reciprocal translocation between the larger end-chromosome of the heterochain and a chromosome not belonging to this chain; probably an inversion had also occurred. Investigation of the genetically aberrant material has shown sure cases of fragmentation: in  $Rn^t Rn^t$  the translocated foreign segment is comparatively often lost, while the small fragment chromosomes in  $Kl$  and trisomic  $C^{pa}$  are also the result of fragmentation. On the other hand, the variation in size of the chromosome 2.3' in the species cross was more consistent with the view that it was the result of exchanges between the chromosomes in a hetero-III.

A few examples of spontaneous chromosome changes that have arisen in species crosses may be given. SVESHNIKOVA (1936) investigated

*Vicia sativa* ( $n=6$ )  $\times$  *V. amphicarpa* ( $n=5$ ).  $F_1$  sometimes formed a hetero-III, the small  $F$  chromosome from *sativa* being paired with the  $A$  chromosomes from the two species. In later generations new chromosomes were observed. One of the  $F_2$  plants had two »unusual» chromosomes, one of them a very small  $F$ , the other an enlarged  $A$ . The new chromosomes must have been formed through an interchange between »non-homologous» chromosomes in the III. Plants in later generations had only one of the changed chromosomes and none of them was homozygous for any of these chromosomes, indicating homozygous lethality. KOSTOFF (1939) found in later generations of amphidiploid *Nicotiana glauca*  $\times$  *N. Langsdorfii* one  $F_3$  plant and one  $F_4$  plant with new chromosomes. He is of the opinion that the new chromosomes have arisen through unequal crossing-over, multivalents being frequent. TOBGY (1943, p. 103) mentions the occurrence of new chromosomes in  $F_2$  of *Crepis neglecta*  $\times$  *C. fuliginosa*. The pairing in  $F_1$  was good but paired chromosomes were very different in size or form; hetero-III's and hetero-IV's were frequent.

The interesting studies of WESTERGAARD (1946) on aberrant forms of *Melandrium* have given examples of chromosomes changed through fragmentation or through exchange of segments. WESTERGAARD (cf. also WARMKE, 1946) has found three different aberrant  $Y$  chromosomes in the offspring of triploid *Melandrium*. One was a terminal fragmentation of the so-called differential arm of the  $Y$  chromosome, one was a fragmentation in the homologous arm, that is, the arm that forms a chiasma with the  $X$  chromosome. The third aberrant  $Y$  was the result of an exchange between the differential arms of  $Y$  and  $X$ . Plants having aberrant  $Y$  chromosomes are intersexual or male sterile.

The frequency of spontaneous chromosome changes is no doubt difficult to estimate. If they are accompanied by phenotypical changes, as in *Melandrium* or  $Kl$  and  $Rn^t$  in *Godetia*, they have a great chance of being detected, if not, the chance is small unless, as in species crosses, many plants are investigated. But even if one takes into consideration these facts, it cannot be doubted that exchanges between chromosomes which are structurally different are the most important cause of the appearance of chromosomes of a new size or form; it seems that such exchanges are facilitated in hetero-multivalents.

The new chromosomes may have unusual properties. In  $Rn^t Rn^t$  a *Whitneyi* chromosome having a translocated *deflexa* segment shows a relatively high frequency of spontaneous changes; the foreign segment is rather often lost. The fragment chromosome carrying  $Cp^a$  is

often eliminated at mitosis, as was shown by green sectors on the seedlings. The small chromosome that had arisen through a fragmentation of the chromosome carrying *G* also seems to be eliminated at mitosis.

### SUMMARY.

The cytological investigation of *Godetia* plants from the cultures of Dr. GUNNAR HIORTH, Vollebekk, Norway, has shown several cases of spontaneous chromosome changes.

In two cases chromosome fragmentation explains the unexpected appearance of the recessive character in heterozygotes, a segment with the dominant allele being lost. A *Whitneyi* chromosome with a *deflexa* segment is rather often fragmented, the foreign segment being lost. A fragment chromosome carrying  $C^{Pa}$  is often eliminated at mitosis.

Dr. HIORTH tried to transfer the petal spot  $F^b$  from *G. amoena* to *G. Whitneyi* by means of backcrossing for successive generations the  $F_1$  hybrid to *G. Whitneyi*. All  $F^b$  plants in the first backcross generation  $F'_2$  were very sterile, and their chromosome pairings showed the presence of several *amoena* chromosomes. Later generations showed different  $F^b$  types. The heterotrisomic type appeared for the first time in  $F'_4$  and had a certain amount of sterility and 6 II + heterochain-of-three. It had 13 *Whitneyi* and 2 *amoena* chromosomes, i. e. the end-chromosomes of the chain. The larger end-chromosome is the  $F^b$  chromosome, but it must be supplemented by the small end-chromosome to give a fertile gone. One trisomic plant had a chain or III consisting of the  $F^b$  chromosome and two *Whitneyi* chromosomes. Still more sterile is a disomic  $F^b$  type. This must have at least three *amoena* chromosomes, for it forms the heterochain and a second configuration-of-three. Exchanges in the heterochain are probably the explanation of the fact that the small chromosome changes its size; in one family it was particularly small. The greater constancy of the heterochain in later generations is perhaps also a result of exchanges. Of rare occurrence is a fertile  $F^b$  type. It has 7 II and a regular meiosis. Here,  $F^b$  must have been transferred through crossing-over to the *Whitneyi* chromosome in the centre of the chain. Thus, the petal spot  $F^b$  is transferred to the species *Whitneyi* by a rare crossing-over in a heterochain. The gene  $C^{Sp}$  from *amoena* is also followed by sterility in similar crosses, and meiosis in an  $f C^{Sp}$  plant in  $F'_2$  showed the presence of a homochain, indicating chromosomal sterility.

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# HETEROCHROMATIC B-CHROMOSOMES IN ANTHOXANTHUM

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## I. INTRODUCTION.

IN a preliminary note by the present writer (1942) on the chromosome numbers in the grass genus *Anthoxanthum* it was reported that some plants of *A. aristatum* had, in addition to the normal 10 chromosomes, also 2—3 extra »fragments».

The population in which this was found was more thoroughly investigated later on, and the results thus obtained will now be described. The possibility suggested by ÖSTERGREN (1945) that such extra chromosomes lead a kind of *parasitic existence* in the populations will also be given a renewed analysis in this connection.

There are many names which have been given to such supernumerary chromosomes as are not homologous with those of the normal complement and as exist in equilibrium in the populations. Naturally, every investigator is at liberty to adopt a name of his own choice for those extra chromosomes which he has discovered. I have »baptized» them *B-chromosomes* in my *Anthoxanthum* case. The reason for this is that they probably constitute a somewhat related case to the supernumerary chromosomes of maize, which are well-known to all geneticists under this name. DARLINGTON and THOMAS (1941) have also adopted this name for the heterochromatic extra chromosomes studied by them in *Sorghum purpureo-sericeum*. The name »accessory chromosomes» suggested by MÜNTZING (1945) and HÅKANSSON (1945) and also used previously in the literature (e. g., COLEMAN, 1943, p. 7) is also a suitable term. »Extra fragment chromosomes», however, is undoubtedly a term which is not appropriate at all for such cases as the present one, as it easily leads to the misunderstanding that the chromosomes have originated from the normal complement by simple fragmentation, a view that can be demonstrated to be wrong.

## II. MATERIAL AND METHODS.

The species of the genus *Anthoxanthum* are typical cross-fertilizers, a fact demonstrated by (1) their flower biology (protogyny), (2) the

high variability of single plant progenies, and (3) the poor seed formation on isolation.

The material of *Anthoxanthum aristatum* containing these B-chromosomes was received from the Botanical Garden of Coimbra, which had collected the seeds on wild plants in Portugal. A comparison of my plants with the descriptions of the Portuguese *Anthoxanthum* species by COUTINHO (1939) shows that they undoubtedly belong to the species *A. aristatum* BOISS.

During the study of meiosis in the plants with B-chromosomes it was found of value to compare the behaviour of B-univalents with that of univalent chromosomes of the normal complement. Such univalents (from non-pairing in normal diploids) are occasionally found in plants of the same material, but to get a bigger material of normal univalents trisomic plants of another *Anthoxanthum* population were studied. In material of *Anthoxanthum aristatum* from the Botanical Garden of Nancy (France) an autotriploid plant was obtained in the progeny of acenaphthene-treated material. This triploid was crossed with diploids of the same population and in this way trisomic plants were obtained.

This *Anthoxanthum* population from Nancy is morphologically very different from the Coimbra material, but the two types are easily crossed and give a hybrid of good fertility, so it is likely that they belong to the same species. This taxonomic question, however, although not very important in the present connection, should be analysed more thoroughly before definite statements are made in this respect. Anyhow, the two types are closely related, and a comparison is therefore justifiable.

The root tips were usually fixed in chrome-acetic-formalin (the NAVASHIN modification by MÜNTZING, 1933, p. 131). For chromosome morphological study root tips of a few plants were fixed in LEVITSKY's fixative (10 % formalin + 1 % chromic acid, equal parts). For study of meiosis the panicles were fixed in chrome-acetic-formalin after prefixing in acetic alcohol (1 : 3). The microtome sections of panicles and root tips were stained with crystal violet or (in a few cases) by the FEULGEN method. The first pollen mitoses were studied in acetocarmine smears and also in material fixed in acetic alcohol, sectioned on a microtome and stained with FEULGEN.

### III. CYTOLOGICAL RESULTS.

#### 1. CHROMOSOME MORPHOLOGY.

A few fixations of root tips from the Coimbra material were made in LEVITSKY's fixative in order to give information concerning the chro-

mosome morphology. Measurements of the chromosomes by the usual method (LEVITSKY, 1931) were undertaken on a slide with  $2n = 10 + 2 B$ . Seven metaphase plates were measured. The chromosomes of the normal complement apparently belong to only two morphologically different types; in the haploid set of five chromosomes there are three with a secondary constriction and two without. The result of these measurements is given schematically in Fig. 2, where a B-chromosome is also depicted. Fig. 1 is a LEVITSKY-fixed metaphase from a plant with  $2n = 10 + 3 B$ . The B-chromosomes in this material belong either to the «standard type» or to the «iso-chromosome type» (cf. below). Which of these types they actually belong to cannot be determined, as only root tips were seen of these plants. The chromosomes of the normal set are about  $6 \mu$  and those of the B type about  $4 \mu$  in length. Of course, it is very likely that the three chromosomes with the secondary constriction are in reality slightly different from one another in morphology and that the same is the case with the other type, but these differences are too small to be ascertained.

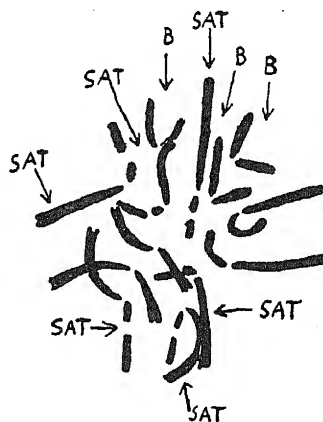


Fig. 1, *A. aristatum*, root-tip mitosis in a plant with  $2n = 10 + 3 B$ . At least 4 of the 6 secondary constrictions are of SAT-type. All chromosomes with secondary constrictions, however, have preliminarily been marked SAT in the figure. Fixative: LEVITSKY. —  $\times 3000$ .

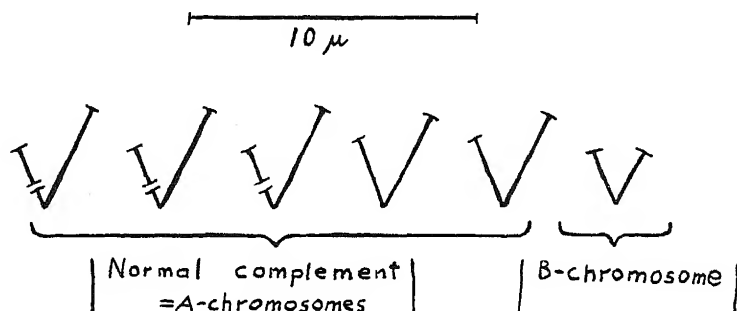


Fig. 2, scheme of the morphology of the haploid chromosome set plus a B-chromosome in *A. aristatum*.

At least two of the three chromosomes with secondary constrictions are SAT-chromosomes, concerned with the formation of nucleoli, as

sometimes 4 nucleoli have been seen in the root-tip nuclei. As, however, nucleoli have a strong tendency to fuse, it is quite possible that all three of them are of SAT-type, and all the chromosomes with secondary constriction have preliminarily been marked »SAT» in Fig. 1.

The numerous fixations in chrome-acetic-formalin, although less clear in this respect, give definite support to the above chromosome morphological scheme.

The chromosome morphology of the Nancy type has not been analysed in detail, but so much is obvious that it is clearly different from the Coimbra material.

## 2. FREQUENCY AND TYPES OF B-CHROMOSOMES.

The *Anthoxanthum* population from Coimbra contained B-chromosomes of the following three types:

(1) »Standard» type. — These chromosomes are about  $4\ \mu$  in length at mitosis and their two arms are of the same length.

(2) *Iso-chromosomes*. — These are morphologically quite similar to the preceding type, but they differ from it in their behaviour during meiosis, where their two arms are able to pair with one another, thus giving ring-shaped univalents.

(3) *Very small chromosomes*. — These have only been seen in meiosis, where they appear as small round dots (Figs. 25—27).

The two first types are rather similar to one another. To distinguish between them requires a careful study of meiosis, and in cases of very strong chromosome contraction even this may not be sufficient, as then the rod univalents may also appear to be round like the rings of iso-univalents. (The chromosome contraction at meiosis is highly variable in this material.) Consequently, it is not possible always to state to which of these two types a B-chromosome belongs.

The standard type seems to be the commonest one. That is why I have called it standard. But it may of course be possible that a study of more extensive material will show that some other type is even more common.

The chromosomes of the third type have been very little studied, so all descriptions below will refer to the first two types, unless otherwise is stated.

The frequency of the various types of B-chromosomes was studied in meiosis of plants derived from the original seed sample, and the result was as follows:

0 B: 12 plants.

1 B: 4 plants. Two of them have standard B's and the two others have B's of the very small type.

2 B: 2 plants. Both have standard B's. One of them had 2 B's in all studied parts, the other was a chimaera having 1 B in one flower and 2 B's in another.

3 B: 1 plant. It seems that all chromosomes are of standard type.

4 B: 3 plants. One seems to have all four chromosomes of the standard type; the second has at least two of iso-type, the rest of them may be iso- or standard chromosomes; the third has at least one iso-chromosome + two of iso- or standard type and its fourth chromosome of the very small type.

Thus, out of 22 plants there were 10 which had one or more B-chromosomes. It may, however, be a little dubious whether this determination is a reliable estimate of the frequency of B-chromosomes in the wild population. Firstly, of course, the number of plants studied is rather small, and, secondly, nothing is known as to whether the seed sample had been collected from many plants or perhaps only from a single one.

However, it is rather likely that they are common. If they were not, there should have been only a small chance of getting, as I did, three different types in so small a sample.

### 3. HETEROCHROMATY OF THE B-CHROMOSOMES.

As the accessory chromosomes of some plants are known to be heterochromatic (e. g., *Zea mays*, *Sorghum purpureo-sericeum* and *Narcissus Bulbocodium*), it was of some interest to see whether this is the case in *Anthoxanthum* as well. My first impression was that it was not, but later on I got clear evidence of the heterochromatic nature of the accessory chromosomes in the present material.

There are some known facts and hypotheses concerning the genetic and physiologic properties of heterochromatin, but it should not be forgotten that heterochromatin is primarily a *morphological* concept, as already pointed out by HEITZ (1928, p. 765) in his original definition of heterochromatin.

Here the slightly improved definition of HEITZ (1932, pp. 626—627) may be given: »Als Heterochromatin bezeichne ich also ein in der Grundsubstanz des Ruhekerns sichtbar vorhandenes, in Bezug auf die Längsrichtung des Chromosoms bestimmt gelegenes Stück desselben, welches mindestens in der Telo- und Prophase,

manchmal auch in der Ana- und Metaphase stärker färbbar ist als die übrigen Stücke, als das die Grundstruktur bildende Euchromatin.»

A fact already recognized in HEITZ's original paper (1928) should also be pointed out, viz. that the heterochromatic behaviour may sometimes be manifested in all nuclei or divisions and that in other cases it may be restricted only to special phases in the life cycle of an organism.

Later on, it turned out that chromosome parts which exhibit overstaining (positive heteropycnosis) at some stage, and thus are in agreement with HEITZ's definition, may still differ from this definition by manifesting an *understaining* (negative heteropycnosis) at some other stage. (The terms positive and negative heteropycnosis are due to WHITE, 1935, p. 66.)

A common view of what heterochromatin is may now be expressed in the words of WHITE (1945): »It will be convenient to use the term *heterochromatic* to describe any chromosomal region which becomes heteropycnotic at some stage in its cycle. Chromosomal regions which never under any circumstances show heteropycnosis may be called *euchromatic*» (l. c., p. 28).

The recognition that negative heteropycnosis should also be ranked among the heterochromatic phenomena may very well make the groups »heterochromatin» and »secondary constrictions» confluent. (Cf. also the speculations of KLINGSTEDT, 1941, pp. 171 and 173.)

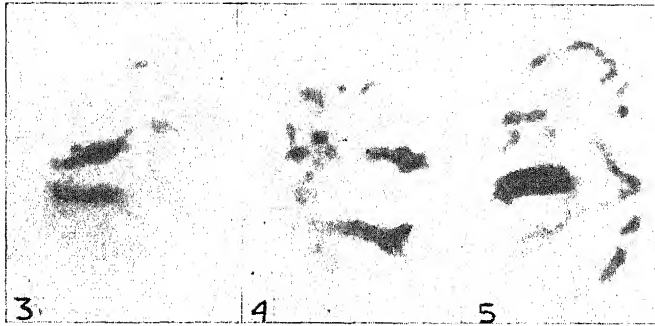
The heterochromatin of my *Anthoxanthum* material has only been studied in the root tips. The normal complement of *Anthoxanthum aristatum* also contains some heterochromatin, forming a number of small overstained bodies in the resting nuclei. In rare cases these may by chance have been brought in a position close to one another, so that they together form a somewhat larger body. In the plants with B-chromosomes, however, larger heterochromatic bodies of this kind are much more common, and they increase in frequency with an increasing number of B's in the plant. It would be expected that these big chromocentres would be equal in number to the B's of the plant; and cells having that number of chromocentres can also be found (e. g., Figs. 3—4). In most cells of plants having more than one B, the number of chromocentres is, however, lower than expected. This is undoubtedly due to a close association of B's with one another, whereby they form joint chromocentres.

In the root-tip prophase the change of these chromocentres into mitotic B-chromosomes can be followed. In the prophase, too, they often show a stronger staining than the normal complement. Furthermore, it can be seen here that the B's quite often lie very close to one another (Fig. 5), an observation that confirms the above view as to the reason the chromocentres in the resting nuclei are often fewer in number than the B-chromosomes at metaphase.

This type of association can also frequently be found within a single chromosome, its two arms associating with one another.

Such a close association of heterochromatic parts is a fact also known from many other organisms. A well-known example is the chromocentre in the salivary gland nuclei of *Drosophila*. GEITLER (1939) also found that the heterochromatic Y-chromosomes in various *Heteroptera* often formed a joint chromocentre in polysomatic nuclei. Fusion of heterochromatic bodies in *Fritillaria* was reported by DARLINGTON and LA COUR (1941).

The degree of heteropycnosis in the resting nuclei is very variable in my material. In some plants it is much more pronounced than in others. Furthermore, there is a very characteristic difference between cells in the same root tip. The heterochromatin is much more easily



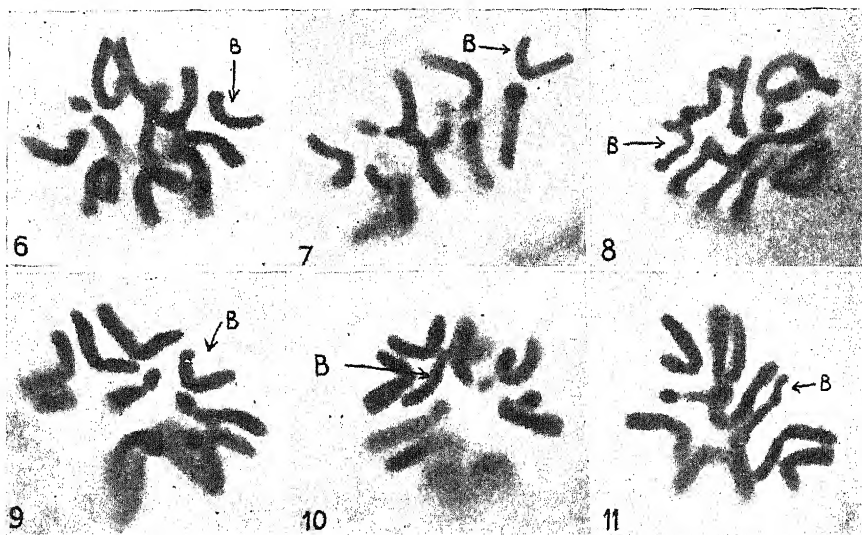
Figs. 3—5, positive heteropycnosis of B-chromosomes in root-tip cells of a plant with 2 B's. — Figs. 3—4, resting nuclei. — Fig. 5, prophase. —  $\times 2700$ .

seen in somewhat older cells (in the higher regions of the root) than in the young vigorously growing meristematic cells.

This difference is not only due to the fact that the euchromatin is more weakly staining in the older cells, but it actually also seems that the B-chromosomes are more diffuse and weakly staining in the resting nuclei of the younger cells than in the older ones.

The B-chromosomes also show a differential pycnosis at metaphase, but then the difference is of the opposite kind, they are narrower than the normal chromosomes (negative heteropycnosis). This is especially clear in cold-treated plants (Figs. 9—11) but a slight difference of this type also exists at normal temperature (Figs. 6—8). It might be suggested that the more pronounced negative heteropycnosis at low temperature is due to a »differential reactivity» of the same type as that found by DARLINGTON and LA COUR (1938, 1940, 1941) for seg-

ments of the normal chromosomes in *Paris*, *Trillium* and *Fritillaria*, by GEITLER (1940) in *Adoxa*, by LEVAN (1942) in *Secale*, and by CALLAN (1942) in *Triton*. The negative heteropycnosis in my case, however, seemed to be visible even at normal temperature, and it is possible that its clearer visibility at low temperature may be due simply to the fact that differences in width are more easily seen when the absolute width of normal as well as B-chromosomes is larger because of the cold contraction. Hence it need not necessarily mean a differential reaction to low



Figs. 6—11, negative heteropycnosis of B-chromosomes in root-tip mitoses of a plant with one B at different temperatures. — Figs. 6—8, ordinary temperature (19° C. at the time of fixation). — Figs. 9—11, cold treatment for four days at -1,5° C. —  $\times 2450$ .

temperature, even if this is a likely interpretation. It should also be pointed out that the negative heteropycnosis is rather variable in my material (like the positive one) and that it is not always easily seen.

There are other cases known of negative heteropycnosis at normal temperature, especially in animals (WHITE, 1935, 1940; GEITLER, 1937; KLINGSTEDT, 1941; and KOLLER, 1938). LEVAN (1942) found that the differential segments of rye which could be made visible at metaphase by cold treatment could also be found at normal temperature in a *haploid* rye plant, and he concludes that the lower viability of the haploid obviously acts here in the same manner as cold treatment (l. c., p. 182).

The B-chromosomes of *Anthoxanthum* do not seem to be heteropycnotic during the meiotic prophase.

It is not known whether there is a difference in heteropycnotic behaviour between the B-chromosomes of standard type and those of iso-type in my material.

#### 4. CONSTANCY OF THE B-CHROMOSOMES.

Some cases are known where the number of accessory chromosomes is changed during the development of the individual. Firstly, there are *Sorghum purpureo-sericeum* (DARLINGTON and THOMAS, 1941) and a type of *Poa alpina* (MÜNTZING, 1946 b), in which plants the accessory chromosomes are regularly eliminated from the somatic tissues of the individuals (or at least their root tips) and only preserved in the germ-track. Secondly, there are cases where the accessory chromosomes seem to show a more general instability in number. This is known, e. g., in *Tulipa galatica* (DARLINGTON, 1937, p. 63, referring to unpublished results of UPCOTT). The B-chromosomes of maize also exhibit occasional changes in number during the development of the plants (DARLINGTON and UPCOTT, 1941, p. 279). In this case it is also possible directly to see the aberrant behaviour of the B-chromosomes during the mitoses. They usually lie at the edge of the metaphase plate and their centromeres may divide either before or after those of the normal chromosomes (DARLINGTON and UPCOTT, l. c.). This is regarded as due to a centromere weakness.

My observations on the *Anthoxanthum* material demonstrate that the B-chromosomes of standard and iso-type (which must be treated together, as they are indistinguishable in many cases) have rather a good constancy in the various parts of the plants; changes in number do occur but are relatively rare.

Thus, in 86 plants in which the somatic number of B's (1—4) was determined, counts were made (in practically all cases) in at least three different roots of the same plant. These three roots gave the same number in all cases with the single exception of an entirely tetraploid root where the number of A- as well as B-chromosomes was doubled. This exception, of course, is not due to aberrant behaviour of the B's. Nor were there ever seen in these mitoses any signs of abnormal behaviour of the B's, such as precocious or delayed centromere division or a delayed congression on the plate.

In 31 plants (13 with no B in the roots and 18 with one or more

B's) the same number of B's was found in both root tips and meiosis. In two plants, however, the number of B-chromosomes differed between root tips and anthers, and in a third case there was a difference between different flowers in the same panicle, one flower having a single B, another having two.

The B-chromosomes of the very small type seem to be more irregular. They show disturbances of congression and probably also of division at the second division of meiosis, and in all the three cases in which root tips and anthers were compared these B's had been eliminated from the root tips. Anyhow, these very small B's have been found to be transmitted from one generation to the next (observed at meiosis in mother and progeny), so they must have a certain degree of stability.

#### 5. BEHAVIOUR AT MEIOSIS.

The chromosomes of the normal complement in *Anthoxanthum aristatum* usually pair as  $5_{II}$  at meiosis. There is often a low frequency of non-pairing in the plants, as is probably found in most organisms. Multivalents have not been found in the about 20 plants examined in more detail. (In this respect this diploid species differs strikingly from the tetraploid *A. odoratum*, where multivalents, even such higher than quadrivalents, are of quite common occurrence; KATTERMANN, 1931; PARTHASARATHY, 1939; and ÖSTERGREN, 1942.)

The behaviour of *normal* chromosomes in excess has been studied in triploid and trisomic plants of the material from Nancy. These chromosomes often form trivalents but are also often left unpaired as univalents (Figs. 12 and 13).

During anaphase of the first division these univalents often lag, and the lagging univalents very frequently divide into two daughter univalents, which are usually included in the telophase nuclei (Fig. 14). In the second division these daughter univalents are unable to divide and they lag between the separating anaphase chromosomes (Fig. 15). In this way they are very often left outside the four normal tetrad nuclei, and form separate micronuclei (Fig. 16), which at later stages can be seen to transform into dark-staining chromatin droplets. Thus, there is a considerable elimination of univalents derived from the normal complement.

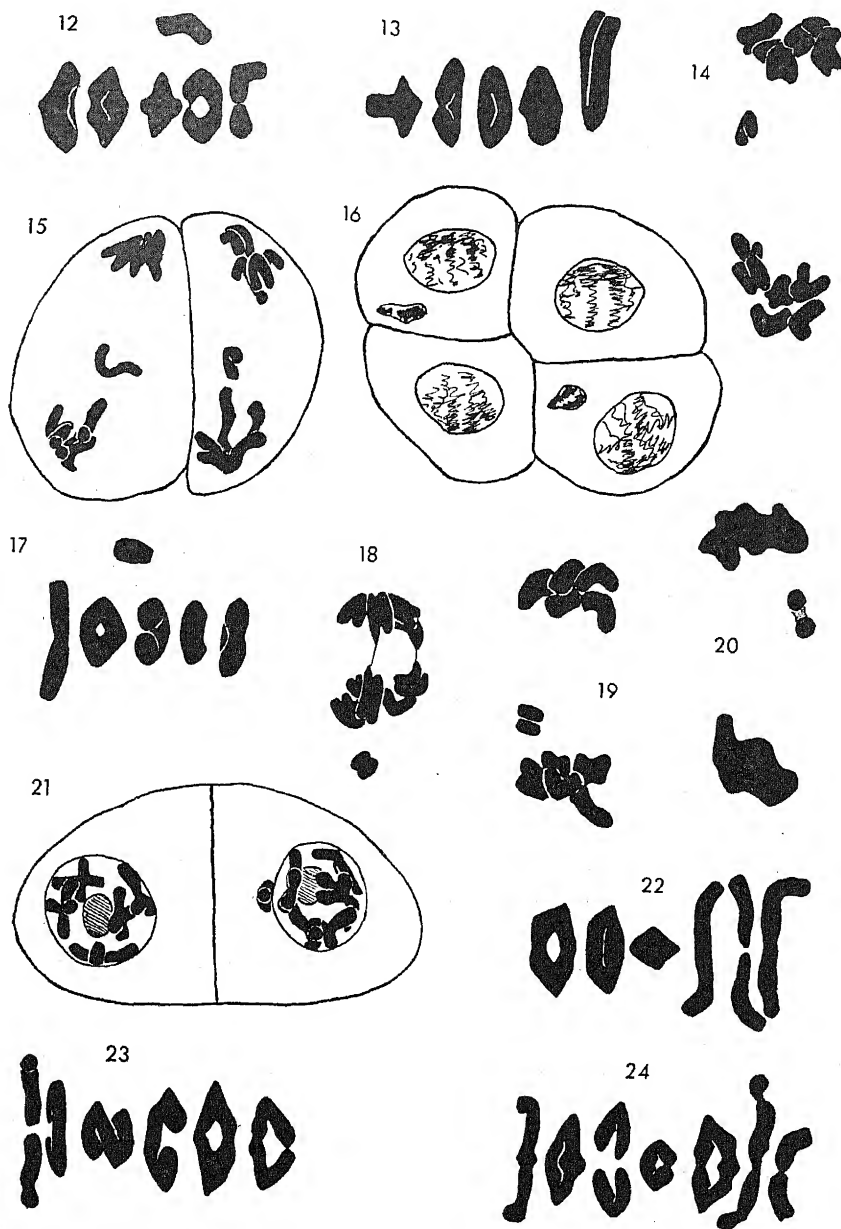
The behaviour of univalent B-chromosomes is quite different from this. They can be studied quite well in plants having a single B-chromosome (of »standard» type).

The extra B-chromosome does not pair with the normal chromosomes. In a plant of this type 208 cells were studied; in 207 of them the B was unpaired and in a single one it was in terminal contact with a rod bivalent, probably forming a trivalent. A great number of other first metaphases were also studied in another plant (without counting them) and the B-chromosome was always unpaired. The single case of pairing with a normal chromosome is not sufficient to be made the basis of a hypothesis of partial homology of the B with this chromosome. It may very probably be a quite unspecific sticking together of heterochromatic parts.

At anaphase of the first division the B-univalents, like the normal univalents, may either be included in one of the polar groups or may lag between them (Figs. 18—19). Here, however, there is a very important difference from the normal univalents. *Lagging univalents of B-type never divide at first anaphase.* They manifest their doubleness (i. e. that they consist of two chromatids) like all the other chromosomes at this stage; a result considered to be due to »lapse of chromatid attraction». But the two chromatids are never found in a stage of moving apart, as is so often observed in normal univalents. The centromere can sometimes be seen to be subjected to tension in the direction of the two opposite poles, but there is some *connecting link* (either in the centromere itself or very close on both sides of it?) which keeps the two halves together. This link is seen to be extended (Fig. 20), but it does not break, so there will be no effective division. Such a lagging B-chromosome may later (in early telophase) be included in one of the two normal nuclei, or it may form a small nucleus by itself, often in close proximity to one of the big nuclei (Fig. 21).

As a consequence of the absence of division at first anaphase, the B-chromosome is able to divide normally in the second division. In the tetrads micronuclei formed by eliminated B-chromosomes are practically never seen. Not a single one was found in 219 tetrads. In a slide from a trisomic plant, on the other hand, a count among 132 tetrads showed 79 with one or more micronuclei and only 53 without — a striking difference.

The non-division of univalent B-chromosomes at first anaphase, whereby they differ from the univalents of the normal complement, appears as a *purposeful adaptation to maintain the B-chromosomes in the population* (cf. below). The case also tells us that a higher frequency of unpairing does not necessarily mean a higher elimination of



Figs. 12—16, meiosis in a trisomic plant (with a *normal* chromosome in excess). — Fig. 12, first metaphase,  $5_{II} + 1_I$ . — Fig. 13, first metaphase,  $4_{II} + 1_{III}$ . — Fig. 14, first anaphase with a dividing univalent (the lower daughter univalent has reached the anaphase group). — Fig. 15, lagging daughter univalents at second anaphase. — Fig. 16, a tetrad with micronuclei formed by daughter univalents.

a chromosome, and thus warns us not to make hasty conclusions in this respect.

In plants with two B-chromosomes of »standard» type these very regularly pair with one another, forming a small bivalent, usually of the ring-shaped type with chiasmata at both ends (Fig. 22). In 54 cells of a plant of this type the frequency of chiasmata at metaphase in the B's was compared with that in the normal chromosomes. The average for the B's was 1,833 per bivalent and that for the normal chromosomes 1,737. The difference is not statistically significant ( $P > 0,2$ ).

The B-chromosomes never had more than a single chiasma in the same chromosome arm, while the normal chromosomes sometimes had two. If, instead of counting the exact frequency of chiasmata, we only count the frequency of paired chromosome arms, a procedure sometimes adopted in such investigations, we shall thus get a different result, viz. 1,833 for the B's and 1,596 for the normal bivalents. Measured by this method the pairing is significantly higher in the B's ( $P < 0,01$ ).

The good pairing of the B's was confirmed (although not quantitatively analysed) in another plant with two B's of standard type. The good pairing of the B-chromosomes is rather surprising; firstly, because they are shorter than the normal chromosomes, and, secondly, because they are heterochromatic. The heterochromatic B-chromosomes of maize show a reduced pairing as compared with the normal chromosomes (DARLINGTON and UPCOTT, 1941). Perhaps the good pairing of the B's in the present case is connected with the absence of heteropycnosis in them at the prophase of meiosis. In tetraploid spermatocytes of a locust WHITE (1933) found that the two X-chromosomes were still heteropycnotic, just as the single X in the normal diploid spermatocytes. These heteropycnotic X-chromosomes were unpaired. In the female meiosis, however, where the two X's are not heteropycnotic or only so to a small degree, they show a good pairing. WHITE concluded that the absence of pairing in the males was due to the heteropycnosis (cf. also WHITE, 1945, p. 89, and COLEMAN, 1943).

Figs. 17—24, meiosis in various plants with B-chromosomes. — Fig. 17, first metaphase,  $5_{II} + 1 B_I$ . — Figs. 18—20, first anaphases in a plant with a single B. — Fig. 18, the univalent has a polar position and will no doubt be included in the anaphase group. — Fig. 19, a lagging B-univalent manifests doubleness but does not divide. — Fig. 20, like the previous figure, but a later stage, a connecting link prevents the chromatids from moving apart (univalent seen in end view). — Fig. 21, prophase of second division, the undivided B has formed a small nucleus close to one of the big nuclei. — Fig. 22, first metaphase,  $5_{II} + 1 B_{IV}$ . — Fig. 23, first metaphase,  $5_{II} + 1 B_{IV}$ . — Fig. 24, first metaphase,  $5_{II} + 2 B_{II}$ . —  $\times 2650$ .

Thus, there is some evidence that the absence of heteropycnosis in a heterochromatic chromosome may favour its pairing.

Consistent with this good pairing of the B-chromosomes is also the fact that they quite commonly form trivalents and quadrivalents in the plants having three and four B's. The frequency of trivalents in the 3 B plant studied is about as high as in plants having three homologous members of the normal complement (trisomics and a triploid), i. e. about 50 %.

This observation is important when we have to consider the homology relations of the B-chromosomes in *Anthoxanthum*. We know that the normal chromosomes have a good ability to form trivalents among themselves when they are three and that the same is the case with the B-chromosomes, and, further, that the B-chromosomes do not pair with the normal chromosomes, not even in plants having only a single B. The conclusion must be that they are not homologous with the chromosomes of the normal complement. (The single cell, out of 200, where there might have been such a pairing between A- and B-chromosomes does not mean very much in this discussion.)

*Iso-chromosomes.* — In one 3 B plant I found that at least one chromosome was an iso-chromosome, there being seen in the univalents a pairing between the two arms of the same chromosome to form rings. Similarly it was found that one 4 B plant must have at least two such iso-chromosomes. Especially this 4 B plant gave very clear evidence in this direction (Fig. 29). These two iso-chromosome plants were also characterized by a higher frequency of univalent B-chromosomes, as should be expected from the fact that, here, an intrachromosomal pairing is competing with the normal interchromosomal one.

It might be suggested that all the B's of this size (groups 1 and 2, see p. 264) might be iso-chromosomes, but this is very unlikely when we consider the very perfect interchromosomal pairing in the 2 B plants. Here univalent B's are completely absent. The different shapes of the univalents in the various plants (rings or rods) also support the present interpretation that there are two kinds, standard B's and iso-chromosomes. This distinction, however, is not definitely established. There may be a single kind of B that behaves differently because of the different physiological conditions in different plants, some conditions in that case favouring a non-homologous pairing (or sticking together of chromosome parts) that simulates iso-chromosome behaviour and others not. FERNANDES (1946) found a considerable degree of non-homologous pairing in a heterochromatic supernumerary chromosome in *Narcissus*.

*Very small B-chromosomes.* — In two plants of the original sample there was found, besides the normal 5 bivalents, a very small univalent

chromosome (Figs. 25—27). Another plant was found to have a probably even smaller univalent besides  $5_{II}$  and 3 of the ordinary B's. This extremely small chromosome was also observed in the progeny of this plant, so it can obviously maintain itself from one generation to another.



Figs. 25—27, meiosis in plants with B-chromosomes of the very small type. — Fig. 25, first metaphase. — Figs. 26—27, second metaphases from two different p.m.c.'s, in Fig. 26 delayed congression of the B.

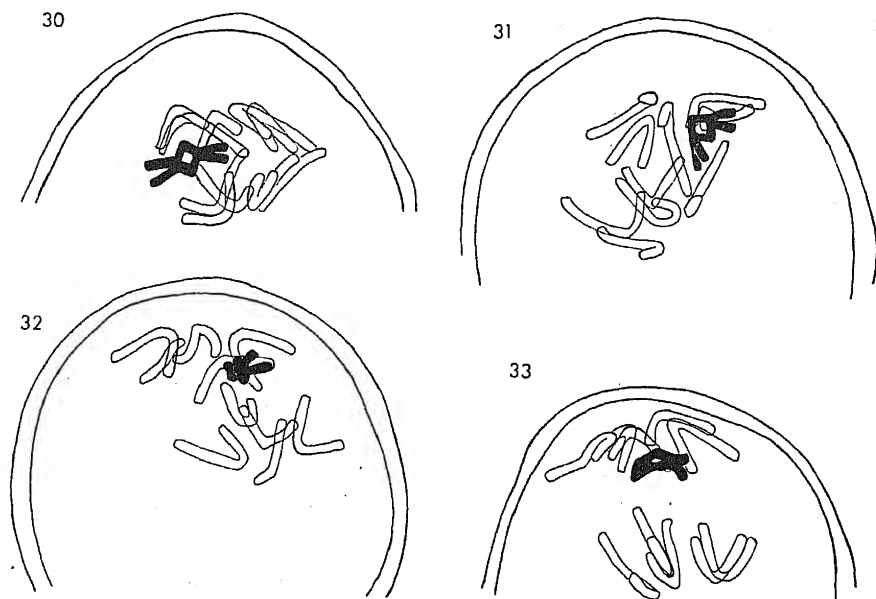
Figs. 28—29, first metaphase in a plant with four B-chromosomes, at least two of which are iso-chromosomes. — Fig. 28,  $4_{II} + 2_I + 1 B_{IV}$ . — Fig. 29,  $5_{II} + 1 B_{II} + 2 B$  ring univalents. —  $\times 3510$ .

These small chromosomes seem to be eliminated from the root tips, as pointed out above. At the first division they lie like ordinary univalents outside the metaphase group, and at the second division they manifest irregularities of congression on the plate (Fig. 26) and probably also of division. Hence they probably have an aberrant centromere.

Very small extra chromosomes are also known in maize, where they are called the F-type (RANDOLPH, 1941, p. 627).

## 6. FIRST POLLEN GRAIN MITOSIS.

Irregularities of behaviour at the first pollen mitosis are known in the case of the accessory chromosomes of *Secale cereale* (MÜNTZING, 1946 a) and *Sorghum purpureo-sericeum* (DARLINGTON and THOMAS, 1941). In both cases the two daughter halves of the B-chromosome fail to separate and are included in the generative nucleus, thus increasing their frequency in the germ-track. In the *Sorghum* case, however, the process is complicated by the occurrence of supernumerary mitoses.



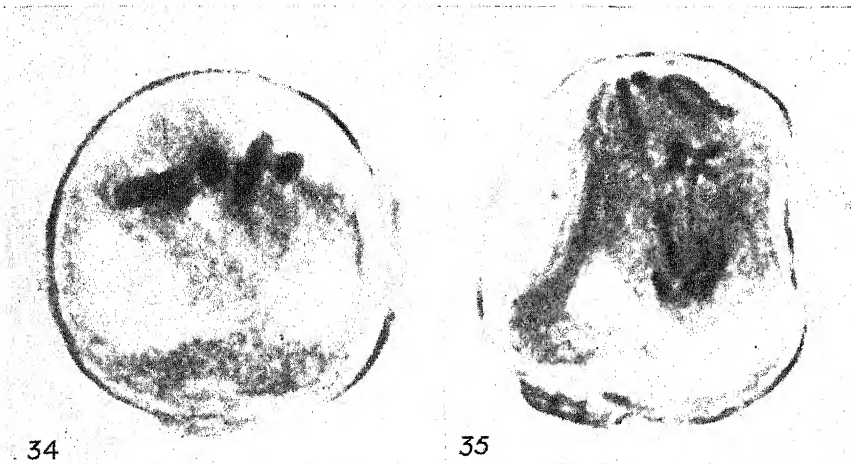
Figs. 30—33, anaphase of the first pollen mitosis, showing non-disjunction of the daughter B-chromosomes. —  $\times 2150$ .

DARLINGTON and THOMAS suggest that the non-disjunction in *Sorghum* is due to an aberrant centromere, a rather likely hypothesis. In the rye case MÜNTZING has demonstrated that the centromere divides normally, but the two chromosomes remain associated in another chromosome region close to the centromere.

In *Anthoxanthum* could also be demonstrated a regular non-disjunction of the two halves of the B-chromosome to the generative nucleus. And my studies also clearly showed that the mechanism is quite the same at that found by MÜNTZING in rye.

At early anaphase we very often find in the B-chromosome plants the quite characteristic chromosome configurations depicted in Figs.

30—33. The B-chromosome is lagging between the two separating anaphase groups of normal chromosomes. It has a characteristic appearance with a central loop and four chromatid arms attached to it. The central loop is kept open by the attractions of the two daughter centromeres to the two opposite poles of the spindle. The B has a strong resemblance to a bivalent at the first metaphase of meiosis with a localized chiasma on each side of the centromere. This configuration demonstrates that the centromeres of the B's divide normally, but the two daughter chromosomes cannot separate because they are attached to one another at one point on each side of the centromere. The un-



Figs. 34—35, photographs of the first pollen mitosis. — Fig. 34, a metaphase in side view showing the asymmetric position of the mitotic figure. — Fig. 35, first anaphase showing non-disjunction of daughter B-chromosomes. —  $\times 2080$ .

divided B-configuration is later included in that anaphase group which is lying close to the wall. (The mitotic spindle has a very asymmetric position in the pollen grain, with one pole close to the wall and the other in the central part of the grain.) This peripheral group is transformed into the generative nucleus and the central one becomes the vegetative nucleus.

The asymmetrical position of the mitotic figure is illustrated by the photograph Fig. 34, showing a metaphase in side view, and the characteristic lagging B-chromosome is given in the photograph Fig. 35. Here the central loop can be seen to be attenuated by the pull of the centromeres.

In other respects the pollen mitoses in these plants usually proceed normally. Normal mitoses were found in 1 plant with 4 B's, 3 plants with 2 B's and 2 plants with 1 B. In a single plant with 1 B, however, there was found lacking differentiation of vegetative and generative nuclei, combined with supernumerary mitoses. As development was normal in the other plants, it is reasonable to suppose that the B was not the chief cause of the abnormality; but it might, of course, have contributed to the effect.

The process outlined above contains two different factors deserving consideration from a mechanical point of view. Firstly, there is the attachment of the daughter chromosomes to one another, and secondly, there is the preferential distribution of the B configuration to the generative nucleus.

Considering the attachment, the first thing to be pointed out is its localized position. It is limited to one point on each side of the centromere. Thus, it cannot be due to a general stickiness of the chromosome, nor to a general delay of the lapse of chromatid attraction. This is clear from the observation that the rest of the chromosome arms are free from one another and often point in different directions. The same conclusion was arrived at by MÜNTZING in his rye case.

As to this localization, two possibilities may be suggested, viz. *either* the factor responsible for it is a *special* (chemically or otherwise structurally) *differentiated segment* localized at the point of attachment, *or* there is no such segment of special constitution here, but the localization of the point of attachment is a kind of *position effect* due to the fact that this region is lying at a certain distance from other regions of decisive importance, such as the centromere or the chromosome ends. (In the latter case the determination of the position of attachment would be determined in a way somewhat related to that for determining the position of localized chiasmata in bivalents.) Even in the latter case there is of course also a special constitutional differentiation of the B-chromosome from the other chromosomes, because the others undergo no non-disjunction, but this special differentiation is then not localized at the points of attachment.

At present we are not in a position to distinguish between these two possibilities. Anyhow, in both cases another factor must also be of decisive importance, viz. a special physiological condition only found during the first pollen mitosis, as that is the only stage in the life cycle at which the effect is manifested. Thus the effect must be the result of an *interaction* between this special physiological condition in the

cell and some special constitutional property of the B-chromosome, whether this is localized in a special region or not.

MÜNTZING (1946 a), from his studies of the same process of non-disjunction in rye, also concluded that it resulted from such an interaction between a special chemical constitution in the fragment and the environment present in the pollen grains (l. c., p. 113). He suggested that there were special, probably heterochromatic segments close to the centromeres which caused the non-disjunction by their delayed reproduction, and also discussed the possibilities that it might be due to a delay of the lapse of chromatid attraction or some kind of stickiness.

I think that it will be impossible to determine at the present stage whether this localized chromatid attachment is due to a delayed reproduction or to some kind of localized stickiness or chromatid attraction.

The regular distribution of the B-configuration to the generative nucleus obviously suggests that the mitotic apparatus must be mechanically asymmetrical in one way or another. But the first pollen mitosis is, indeed, already known as a very asymmetrical mitosis. Not only are the two nuclei to which it gives rise very different from the very beginning, but the asymmetry is also often manifested in the shape of the spindle already at metaphase (GEITLER, 1935, p. 370). One pole is in contact with the cell-wall and is blunt, and the other is in the central part of the grain and is more pointed. I have found an asymmetry of the spindle of this type in the present material as well. The mitotic figure, here, is also asymmetrical in such manner that the metaphase plate is not lying half-way between the two poles but is closer to the generative pole. At the anaphase the generative chromosome group moves only a short distance and the vegetative one a longer distance. Undoubtedly the asymmetry must be an important factor in this mechanism of directed non-disjunction, even if the details of the process are not yet clear.

*Delayed pollen mitosis by B-chromosomes.* — It has already been found by DARLINGTON and THOMAS (1941) and MÜNTZING (1946) that pollen grains having accessory chromosomes divide later than the normal pollen grains in the same anther. I could demonstrate the existence of the same phenomenon in my material.

This phenomenon was studied in two plants with  $2n = 10 + 1 B$  and in one plant with  $2n = 10 + 4 B$ . The time of development of the anther was estimated (as was done by the authors cited) by counting the frequency of binucleate pollen.

The 1 B plants had, as expected, two kinds of pollen:  $n=5$  and  $n=5+B$ , the 4 B plant had in most cases  $5+2B$ , but also  $5+1B$  (from a 3 to 1 distribution at first anaphase) and in two cases only  $n=5$ .

TABLE 1. *The correlation between the number of B-chromosomes at the first pollen mitosis and the average number of nuclei per pollen grain in the anther.*

Chromosome number of plant	Total grains	Number of B's = $y$			Total B's	Average of B's per mitosis	Average of nuclei per grain = $x$	Number of grains for det. of $x$
		0	1	2				
10 + 1B	4	—	4	—	4	1,000	1,940	116
	4	—	4	—	4	1,000	1,848	112
	29	6	23	—	23	0,793	1,690	87
	16	4	12	—	12	0,750	1,698	112
	24	9	15	—	15	0,625	1,560	91
	32	11	21	—	21	0,656	1,507	75
10 + 4B	30	—	—	30	60	2,000	1,634	205
	29	—	1	28	57	1,966	1,453	95
	40	1	4	35	74	1,850	1,423	111
	31	1	30	—	30	0,968	1,039	206

Each line represents an anther.

The observations are given in Table 1. The material was statistically tested by the method of analysis of co-variance (BONNIER and TEDIN, 1940; MATHER, 1943). As the  $y$ -variate I used the number of B's of the individual pollen mitosis. This was correlated to the mean number of nuclei in the pollen grains of the anther ( $=x$ ). The effect investigated was isolated as the joint regression for the two classes 1 B and 4 B. The analysis of variance of Table 1 is as follows:

Item	Sum of squares	N	Mean square	Probability
1. Between classes (1 B and 4 B)	56,39	1	—	—
2. Between anthers within classes	23,41	8	—	—
2 a. Joint regression . . . . .	20,44	1	20,44	< 0,001
2 b. Variation not due to joint regression . . . . .	2,97	7	0,42	0,01—0,001
3. Within anthers . . . . .	29,64	229	0,13	—
4. Total . . . . .	109,43	238	—	—

The error to which the joint regression should be compared is the item 2 b. This item contains, besides variation from random sampling, also such variation as differences between the regressions of the two classes and deviations from linearity of the regressions. It is *a priori* extremely likely that both these kinds of variation exist in such a material, but they are not very interesting in the present connection, so no effort has been made to analyse them.

Anyhow, it is clear enough that there is a regression of the number of B-chromosomes in the mitoses on the frequency of binucleate pollen in the anthers, which demonstrates that pollen grains with B-chromosomes develop more slowly than such without.

The observations on the pollen mitoses do not suggest that there would be a more pronounced elimination of pollen grains having B-chromosomes. But, of course, the delayed division of B-pollen may be the expression of a lowered vigour, which may be manifested also later, e. g., at pollen tube growth. It is not known whether the reduced pollen fertility observed in B-plants affects B-pollen more strongly than normal pollen.

#### IV. INHERITANCE OF B-CHROMOSOMES.

The inheritance of the B-chromosomes has been studied on progeny after *open pollination* of the original plants. The results should be supplemented by studies of controlled crosses, but the present observations also allow of some conclusions. The data are in agreement with the cytological observation of the non-disjunction of B's at the first pollen mitosis and they also demonstrate that *there is no such non-disjunction on the female side*; the effect is restricted to the pollen.

When the results submitted below are being considered it should be borne in mind that the number of individuals in the population containing the mother plants was relatively small, and that these plants did not flower all at the same time, and also that, because of the spatial conditions in the field, the different plants must have had variable possibilities for fertilizing certain other plants. Consequently, it is not justifiable to believe that the population of pollen grains which fertilized these studied mother plants was the same in all cases. Some mothers may have been fertilized chiefly by fathers with B-chromosomes, others by fathers without.

The results are summarized in Table 2, which is based on counts

TABLE 2. *Inheritance of B-chromosomes after open pollination.*

mother	Number of B's of p r o g e n y					Number of plants per progeny
	0	1	2	3	4	
0	7		4			11
0	4		3		1	8
0	1					1
0	5		2			7
0	4	1	3			8
0	3		2			5
0	4					4
0	3					3
1	62	23	2			87
1—2 <sup>1</sup>	19	17		1		37
3		14	9	1		24
4			1		2	3
Total for 0 B mothers	31	1	14		1	
Total for B mothers	81	54	12	2	2	

<sup>1</sup> A chimaera.

of root-tip mitoses of plants the mothers of which were chromosome-counted at meiosis. The progenies from mothers without B-chromosomes must have received their B's from the pollen parent. The B-chromosomes are present in even numbers, 2 or 4, as expected from the cytological observations on the pollen mitosis. The exceptional plant with a single B may be due to a change in the chromosome number during the development of the plant, as such changes have been found to occur sometimes, or it may also indicate that the mechanism of non-disjunction in the pollen mitosis may sometimes fail.

Relatively big progenies were raised from three mothers having from 1 to 3 B's, and a small one from a plant having 4 B's. The three first mentioned progenies must have originated after pollination with pollen having only rarely B-chromosomes. This follows from a combined consideration of *firstly* the behaviour of B's in the pollen (known from the progeny of 0 B-mothers and from cytological studies) and *secondly* the chromosome numbers in these progenies. Thus the progeny after the 1 B mother consist nearly exclusively of 0 B and 1 B

plants. If the pollen had often contained B-chromosomes, we should often have had higher chromosome numbers. This should also have been the case if such a non-disjunction of B's occurred in the embryo-sacs too. Consequently, we can conclude that there is no such non-disjunction on the female side. The table indicates that there has also been a few cases in these progenies where the pollen has transferred B-chromosomes; at least this is a very probable suggestion.

The progenies of the 1 B- and 3 B-mothers should have given a 1 : 1 segregation into respectively 0 and 1 B, and 1 and 2 B. There is however in these cases a conspicuous deficit in the class with the higher number of B's. As the B's are not eliminated during the meiosis (seen in p.m.c.'s), the most probable explanation is a reduced viability of either embryo-sacs or embryos containing B-chromosomes. It should be mentioned that the seeds were stored from 1942 to 1946 before they were germinated, and the germination of these seed-samples was in many cases far from good. There may have been a higher frequency of B-individuals among the non-germinating seeds. (An alternative suggestion would be that the B's may partly be eliminated in the female meiosis although they are not during the corresponding male division.)

In the progeny of the 4 B-mother I got only 3 plants, presumably because of poor germination (there was, however, no exact record made of germination percentage). The two plants with four B's have most probably received two of their B's from the pollen parent.

(The reason there are so few plants in the progenies of 0 B-mothers is chiefly that it was thought better to have a few plants in many progenies than to have many plants in a few progenies.)

## V. PHENOTYPIC EFFECTS OF B-CHROMOSOMES.

There have not been observed any specific morphological features by which the B-chromosome plants differ from the others. It would, however, be very difficult to recognize such features, because the *Anthoxanthum* population in which they exist is so highly variable even without the influence of the B's (0 B plants). Such a high variability is also characteristic of the other *Anthoxanthum* populations I have studied, and hence it need not be connected in any way with the presence of B-chromosomes.

In general vigour as well as in pollen fertility the B plants show a slight reduction as compared with normal plants.

## 1. POLLEN FERTILITY.

The frequency of morphologically poor pollen was counted in pollen slides with the pollen mounted in a mixture of equal parts of acetocarmine and glycerine (a technique often used at this Institute).

TABLE 3. *Influence of B-chromosomes on pollen fertility.*

Number of B's	Percentage of poor pollen											n	Mean %
	0	10	20	30	40	50	60	70	80	90	100		
0	61	8	3	1	1							74	7,84
1	24	7	5	2	1	1					1	41	15,00
2	12	2	2									17	10,29
3				2								2	(35,00)
4			1	1								2	(30,00)

Total number of plants 136.

In each slide (with very few exceptions) exactly 200 grains (poor + good) were counted and the number of poor grains was recorded. The plants studied were from the same progenies as used above to observe the inheritance of the B's. The results are given in Table 3. For testing the results, the  $\chi^2$  method was applied to the data after reducing them in the following way.

	Poor pollen	
	< 10 %	> 10 %
Without B's .....	61	13
With B's .....	36	26
$\chi^2 = 9,794; (0,01 > P > 0,001)$		

Thus, there are fairly good reasons to conclude that the B-chromosomes cause a reduced pollen fertility. In Table 3 there is also a difference between plants with odd and even numbers of B-chromosomes, those with an odd number have a more reduced fertility, but the material is not sufficient to make this difference certain.

## 2. GENERAL VIGOUR.

The plants were classified with regard to general vigour on an arbitrary scale from 1 to 10 by inspection. This procedure was highly facilitated by the fact that the plants were cultivated in readily movable pots, so that it was possible to put any plant in a position between any two other plants to compare it closely with them in order to test whether it might be considered intermediate in vigour.

TABLE 4. *Earliness, vigour and B-chromosomes.*

Degrees of earliness (1 = latest, 13 = earliest)												
1	2	3	4	5	6	7	8	9	10	11	12	13
0;1	0;3	1;3	1;3	1;3	0;3	1;2	0;3	0;3	0;3	1;2	0;3	0;3
0;2	2;3	0;3	1;3	1;3	0;3	0;3	0;3	0;3	1;3	1;4	1;3	0;4
1;3	0;4	1;3	0;3	0;3	0;3	0;3	0;3	1;4	0;3	0;4	0;3	0;5
2;3	0;4	1;4	0;3	0;3	0;3	0;3	0;3	0;4	1;4	1;4	0;6	0;5
0;3	0;6	2;4	0;4	0;3	0;4	0;4	1;4	1;5	1;4	1;4	0;6	4;10
0;3	0;6	1;5	0;4	0;4	0;4	1;4	1;4	1;5	1;4	1;5	0;6	
1;4	1;6	0;5	0;5	1;4	0;4	0;4	0;4	0;5	0;4	0;6	1;7	
1;4	2;7	0;5	0;5	0;4	1;4	0;4	1;4	0;6	0;5	1;6	0;7	
1;4		1;6	0;5	1;5	1;5	0;5	0;5	2;6	1;5	0;7		
0;6		0;6	0;5	0;6	1;5	1;5	2;5	2;7	1;5	0;7		
2;6		0;6	0;6	0;6	0;6	0;5	1;5	2;7	0;5			
0;6			0;6	0;6	1;6	0;7	0;5	1;7	1;5			
2;7			1;6		0;6	0;7	1;6	0;7	0;6			
			1;7		1;7		2;6	3;8	3;6			
			0;9		2;7		0;6	4;8	0;6			
					0;8		0;7	0;9				
							1;7	0;9				
							1;7					
							0;7					
							1;7					
							1;8					
							0;9					

1 square = 1 plant.

Left: number of B's.

Right: degree of vigour.

(1 = strongest,

10 = weakest).

The population studied is, however, extremely variable in earliness. Some plants have ripe seeds at a time when others have not yet started to flower. Obviously it was impossible to compare the vigour of plants so different in their stage of development. For this reason a »two-dimensional» sorting of the plants was applied. They were first sorted in an »east-western direction» by moving the pots into rows of similar earliness, as many rows as could be distinguished (= 13). Afterwards they were sorted inside these rows in a »north-southern direction» according to vigour. The most vigorous plant was put at one end of

each row and the least vigorous at the other, the rest being placed in various intermediate positions according to their appearance. Thus, the difficulties connected with the variation in earliness were overcome by classifying the vigour within classes of earliness. The plants were given numbers on an arbitrary scale, plants appearing to have the same vigour in a row being given the same number, an endeavour being made at the same time to employ numbers having as similar a value as possible in different groups of earliness.

The result of this classification is given in Table 4. Each square represents a plant and the columns correspond to the rows of varying earliness. For each plant is given the number of B-chromosomes (left) as well as the degree of vigour (right).

The effect of different number of B's on the vigour was statistically tested by analysis of covariance as the joint regression within earliness groups.

*Analysis of variance of vigour in Table 4.*

<i>Item</i>	<i>Sum of squares</i>	<i>N</i>	<i>Mean square</i>	<i>Probability</i>
1. Between groups of earliness	54,11	12	—	—
2. Between groups of B's within groups of earliness . . . . .	73,87	22	3,36	Insignificant
2 a. Joint regression . . . . .	20,16	1	20,16	0,01—0,001
2 b. Differences between individual regressions . . .	44,17	12	3,68	Insignificant
2 c. Deviation from linearity	9,54	9	1,06	Insignificant
3. Within groups of B's within groups of earliness . . . . .	335,59	130	2,58	—
4. Total . . . . .	463,58	164	—	—

The result strongly indicates that the vigour of the plants is reduced by increase in the number of B-chromosomes.

The material of Table 4 might also be used to study a correlation between earliness and number of B-chromosomes within classes of vigour. However, such a procedure is a little dubious, as because of the nature of the material it cannot be claimed that the classes of vigour given the same number really correspond when they are within different earliness groups. Anyhow, an analysis of variance of this type was performed on Table 4 and it failed to give any clear evidence of a correlation.

## VI. DISCUSSION.

Supernumerary chromosomes or chromosome fragments have been found in many different species. Undoubtedly they are of different nature in different cases. The simplest cases are those in which the supernumeraries are only duplications of normal chromosomes or chromosome parts. Such arise now and then in most organisms and are soon eliminated because of the genetic unbalance they cause.

Of more interest are those cases in which the extra chromosomes are not homologous with members of the normal complement, and thus cannot be newly formed from that. They have the ability to maintain themselves in the populations by special means (which in some cases are largely unknown) and obviously they must have done so for a very long time. Such chromosomes are known from *Zea mays* (e. g., RANDOLPH, 1941), *Sorghum purpureo-sericeum* (DARLINGTON and THOMAS, 1941), *Secale cereale* (MÜNTZING, 1943, 1944, 1945, 1946 a), *Crepis syriaca* (CAMERON, 1934), *Narcissus juncifolius* and *N. Bulbocodium* (FERNANDES, 1939, 1943, 1946), and *Godetia nutans* (HÅKANSSON, 1945). Probably many other less investigated cases of »extra fragments» also belong here; a list of »supernumerary fragment chromosomes» is given by DARLINGTON (1937, p. 145).

A discussion of such cases is given by HÅKANSSON (1945) in connection with his results from *Godetia*. Characteristic features of them are: (1) Absence of pairing with the normal chromosomes, (2) genetical inertness or subinertness, (3) they are often heterochromatic, (4) »defective» centromeres, causing change in number at mitosis, (5) frequently bad pairing at meiosis, (6) their ability to maintain themselves in the populations. Not all of these features, however, are present in all the cases; thus some cases are not heterochromatic (*Godetia* and *Secale*) and some do not have defective centromeres (especially *Secale*).

Undoubtedly the B-chromosomes of *Anthoxanthum* also belong here. They are not homologous with the normal chromosomes, they are subinert and heterochromatic, they maintain themselves in the population. Their centromeres, however, are but little defective (they may be more defective in the very small type), and they show a surprisingly good pairing with one another at meiosis. In the *Crepis syriaca* case CAMERON also found a very good pairing (1934, p. 267).

From the fact that the extra chromosomes remained in the populations it was concluded by DARLINGTON and UPCOTT (1941) and DARLINGTON and THOMAS (1941) that they were useful to the plants.

If they had not been useful, they would have been eliminated by natural selection.

Another suggestion concerning their function was made by ÖSTERGREN (1945), who suggested that they could quite well remain in the populations without being useful in any way to the plants. The decisive factor for the selection of them would rather be whether they were *useful to themselves*. They are probably in many cases leading an exclusively *parasitic existence* in the plants. It is known from MÜNTZING's observations (1945) that in *Secale* they performed a directed non-disjunction to the generative nucleus during the first pollen mitoses, thus increasing their frequency in the germ-track. This mechanism would cause natural selection to work on them in a way quite different from that on the normal chromosomes. While the normal chromosomes can increase only by being useful, the accessory chromosomes can increase quite irrespective of this by their special mechanism of multiplication. Although, of course, such a mechanism is different in its nature from the spread of parasites by infection, it will in practical results be something closely similar to infection. Accessory chromosomes with a more efficient mechanism of accumulation will be selected, and hence their evolution will tend to perfect this mechanism, and to decrease the factors causing elimination. As these mechanisms may be influenced by genes (e. g., those controlling the course of the first pollen mitosis), the accessory chromosomes will tend to accumulate a system of genes favouring their accumulation. The normal complement, on the other hand, may tend to accumulate genes favouring the elimination of B-chromosomes, so there may result an antagonism similar to that between a parasite and its host. They should also evolve to damage the host plant as little as possible and thus tend to become »inert» or »subinert». (For details I must refer to the original paper.)

The most important feature of such accessory chromosomes is their mechanisms of increasing in number. Theoretically, the same purpose may doubtless be gained in many different ways; but in all cases they must behave differently from the normal chromosomes at some stage or other in their life cycle. In *Anthoxanthum*, as well as in *Secale*, the differential mechanism consisted in a special behaviour of certain chromosome regions close to the centromere, where the daughter chromosomes attach themselves to one another. In *Secale* this mechanism functioned equally well in the female gametophyte as in the first pollen mitosis. In *Anthoxanthum*, however, it functioned only in the pollen. The *Anthoxanthum* case also differs from *Secale* in the absence of B-chro-

mosome elimination at meiosis, caused by the fact that here these chromosomes refuse to divide at the first division and divide regularly at the second. This latter mechanism most probably indicates that they have a centromere different from the normal chromosomes, as normal univalents divide at the first division. (An alternative suggestion might be that, here also, the same mechanism of non-disjunction functioned as is known from the pollen grains. It would be very difficult to recognize such a mechanism here because of the strong chromosome contraction at meiosis.)

In *Sorghum*, too, there is such a non-disjunction to the generative nucleus in the pollen mitosis. This is regarded by DARLINGTON and THOMAS as due to an aberrant centromere. Thus, the mechanism is thought to be different here from that later found in *Secale*, a suggestion that is of course quite possible. Here the chromosomes are also eliminated from the somatic tissue by the same centromere »defect», a mechanism which is useful to the B-chromosomes, as it will reduce the damage to the plant. In *Sorghum*, as in *Anthoxanthum*, there is no elimination of B-chromosomes at meiosis.

The maize data of RANDOLPH (1941) also strongly suggest that in this case, too, there may be some similar mechanism of non-disjunction in the pollen (l. c., pp. 618—619). Here, just as in *Anthoxanthum*, the special mechanism seems to work only on the male side.

In *Crepis syriaca* CAMERON also reports a clear tendency of the extra chromosomes to increase in number in the progeny. To explain this he assumes that these chromosomes may be favourable in one way or another and that consequently they may be selected in gametes or zygotes. Seen in connection with the present results, it seems, however, more likely that they have a special mechanism to produce an increase in number irrespective of selection.

Concerning *Godetia nutans*, HÅKANSSON also stresses the absence in the progenies of any tendency of the accessory chromosomes to decrease. Here, as in *Anthoxanthum*, the B-univalents do not divide at the first meiotic division, and they are practically not eliminated during meiosis. Whether they also have a mechanism leading to an actual increase of them is not known.

Some cases have been reported in which the B-chromosomes seem to have »weak» centromeres, causing them to behave irregularly at mitosis (e. g., *Tulipa galatica* and also *Zea mays*; DARLINGTON and UPCOTT, 1941, p. 279). I think that the abnormal behaviour during the mitoses is due to the same differentiation of the centromere of the B-

chromosomes as enables them to increase their frequency by non-disjunction at some suitable moment in their life cycle. The risk of mitotic elimination is the price which these chromosomes must pay for the favour of being able to increase mechanically in number. Although the centromere may be »weak» from a mechanical point of view, this specialization may in reality be a *strength* of the B-chromosome from a genetical point of view. An extreme case of such variation is described in *Ranunculus acris* by LANGLET (1927), where the number of extra chromosomes varied in a single root tip between 2 and 10, with an average of about 6. Such a variation during the development of the individual might in some cases even be a sufficient means of increasing the frequency of extra chromosomes, if the changes in number occur in an opportunistic way so that gains are more common than losses for the germ-track. It may be of some significance that in *Secale*, where the accumulation mechanism is not the centromere but another chromosome region, the »fragments» show a great stability at mitosis.

According to my interpretation, the equilibrium of such chromosomes in the populations would consist in a balance between a mechanical increase in number and a selective decrease caused by the harmful effects on vigour and fertility which they cause.

To test this hypothesis it will be very valuable to have exact determinations of the efficiency of the accumulation mechanisms of the B's and also to have exact information concerning their phenotypic effects, data which as yet may be insufficient.

The only phenotypic effects actually observed from B-chromosomes are deleterious ones. In maize these chromosomes, when few, have no visible effects. In higher numbers (more than 10—15) they cause reduction of fertility and vigour (RANDOLPH, 1941). In rye, as well as in *Anthoxanthum*, these effects are demonstrable even in plants with a much lower number of accessory chromosomes (MÜNTZING, 1943; and the results above). In *Crepis syriaca* CAMERON (1934) reports reduced fertility and morphological abnormalities from the extra chromosomes. In *Sorghum* they cause reduced pollen fertility and a cytological abnormality called »morbid mitosis» (DARLINGTON and THOMAS, 1941).

The beneficial effects sometimes assumed (CAMERON, 1934; DARLINGTON and UPCOTT, 1941; DARLINGTON and THOMAS, 1941) are as yet exclusively hypothetical.

The strongest support for beneficial effects of the B-chromosomes in maize is furnished by the observations on their inheritance given by DARLINGTON and UPCOTT (l. c., p. 290). The strong loss of the B's

observed there certainly indicates that there must be a positive selection counteracting this loss, and in my paper of 1945 I, too, endorsed the possibility of their being useful (l. c., p. 163).

When studying the data of RANDOLPH (1941), however, we do not at all find such pronounced losses in the progenies of plants with few B-chromosomes. (Only progenies of plants with few B's will be important, because many B's will only very rarely be found in the same plant in populations.) They seem here to be transferred in practically constant frequency. This makes me believe that quite possibly the B's of maize are also parasitic. Now, the B-chromosomes are known to pass through chromosome structural changes rather often. Probably different breeding results can be obtained by employing different types of B's. RANDOLPH (1941, p. 613) reports that POWERS and DAHL had obtained breeding results quite different from his own with their material. I think such differences may be due to this cause. It should be observed that if various mutant B's are found to be eliminated, this is a fact of but little interest in the present connection.

In *Secale*, *Sorghum* and probably *Crepis* the accumulation mechanism seems to have a sufficient efficiency for my hypothesis. *Anthoxanthum* requires further investigation before any statements can be made on this point, but most probably this is the case here, too.

There is a point in my discussion of 1945 which I should like to improve. On pages 160—161 I suggested that if there arose valuable properties in an extra chromosome these would most probably, sooner or later, be »stolen» by the normal complement and thus lose their importance for the B-chromosome (see the original for details). However, this process will only occur if the B-chromosome has a weak mechanism of accumulation. If the B-chromosome possesses a strong ability to increase its frequency, the reverse process may follow. By the mechanical increase of the B-chromosomes the population will get an *overdosage* of the gene or chromosome segment in question. Consequently, selection will favour a decrease of dosage of this gene in the normal complement, and the plant may in this way become dependent on the B in order to get its need satisfied in this respect.

Something similar may also occur in other cases where the B-chromosomes do not contain any special favourable genotypic components, by simple adaptation of the population to the B-chromosomes. This is illustrated by Fig. 36. Curve I represents the relation in a population which has newly been infected by B-chromosomes. Obviously genes will be selected in the population which make B-chromo-

some plants more fertile and more vigorous than they were originally: the plant will tend to adapt itself to the B's. When the B's in this way turn less harmful they will increase in number, because it was

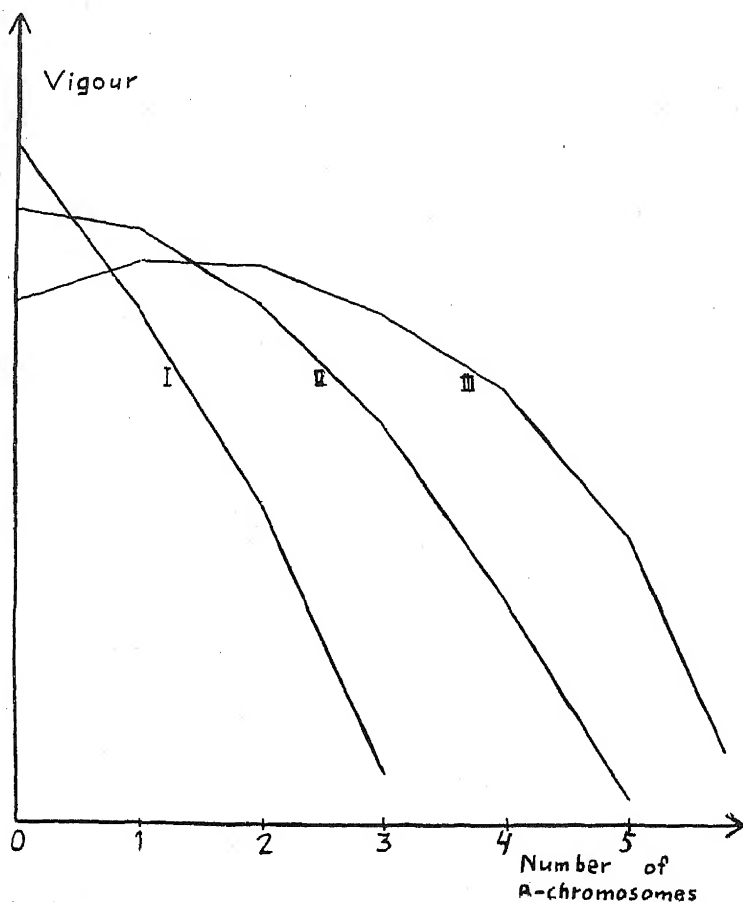


Fig. 36. Hypothetical evolution of a population which becomes infected by B-chromosomes. I = the effect before the population has changed, and II and III various degrees of adaptation of the plant to the presence of B-chromosomes.

their deleterious effects which previously prevented them from increasing. The curve has changed to II in Fig. 36. This will cause a need for further adaptation, and the curve may change to III, etc. As the gene balance in the original population without the B's was the optimal which natural selection could produce in the population, it is likely that the change in balance required for the adaptation to the B's will also cause the plants without B's to be less well adapted. Thus we could

get a situation where plants with a few B's were more successful than plants without B's; but it would be a mistake to conclude from this that the B's had improved the population by their presence.

LONGLEY (1938) had found that maize races with a high frequency of B-chromosomes had a lower frequency of heterochromatic knobs in the normal chromosomes. This may be caused by a mechanism of the type just outlined. The B-chromosomes may impose themselves on the population and, as they produce an overdosage of heterochromatin, the normal complement may have been forced to reduce its heterochromatin. Thus, it need not be concluded from such observations that the B's are useful.

It has been suggested by MATHER (1944) that the apparent inertness of accessory chromosomes is not due to a real absence of genic activity, but that it is caused by the fact that the various genic activities in the B-chromosomes together form a balanced system. This idea is supported by the fact that there seem to exist certain *standard types* of accessory chromosomes. Thus, the extra chromosomes of rye are closely similar to one another in the most various populations (MÜNTZING, 1945, pp. 471—472). LONGLEY (1938), who studied the B-chromosomes of maize from 14 races, found them with a single exception identical in appearance. As the B's retain their type in spite of the fact that aberrant extra chromosomes are very often formed, I consider that there must obviously exist a certain standard type that has a selective advantage. RANDOLPH (1941, pp. 628—629) got the impression that a B-chromosome fragment (called D) might have stronger deleterious effects than the B itself. All this favours the idea that they are not actually inert but that their genic activities together form a harmoniously balanced system somewhat similar to that of a whole set of normal chromosomes. This system, I think, would be selected in such a way that it favours, as far as possible, the accumulation mechanism of the B's and otherwise that it injures the plant as little as possible.

Nothing is known concerning the origin of such B-chromosomes. They seem, however, to be well differentiated from the normal chromosomes. RANDOLPH (1941) in his careful study failed to find any support for the idea that the B-chromosomes of maize could be directly derived from the normal chromosomes. Such extra chromosomes can quite well be very old. They may very well have existed in the ancestors of *Anthoxanthum aristatum* so far back as when these plants did not belong to the genus *Anthoxanthum*.

It is also possible that such parasitic chromosomes may spread

from one species to another by species hybridization followed by back-cross of the hybrid to that species which, previously, was free from parasitic chromosomes. This may be an important factor in Gramineae, where even very widely separated species may cross, such as maize and sugar cane (JANAKI-AMMAL, 1941).

Anyhow, such accessory chromosomes must have originated at some time. It is not likely that a successful B-chromosome can originate from a normal chromosome by a single change (mutation or structural re-arrangement); probably several mutations must have occurred simultaneously or nearly so. The probability per generation of such an occurrence is extremely small, but, in view of the fact that there have been millions of generations available in the past, the chance of such an occurrence will be much greater. When once they have originated they can easily evolve further and differentiate from one another, giving various other types of B-chromosomes, and also most probably they can extend their distribution by also infecting other species by species hybridization.

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### SUMMARY.

A population of *Anthoxanthum aristatum* has been found to contain a variable number of extra chromosomes besides the normal diploid chromosome complement of 10 chromosomes. These B-chromosomes are heterochromatic. They do not pair with chromosomes of the normal complement, but they show a very good pairing with one another. Univalent B-chromosomes are not eliminated at meiosis, differing in this respect from univalents of the normal chromosomes. At the first pollen mitosis the two daughter halves of the B-chromosomes remain attached to one another and are both included in the generative nucleus, thus increasing their frequency in the germ-track. The inheritance of B-chromosomes demonstrates that such a non-disjunction occurs only in the pollen but not in the embryo-sac. The B-chromosomes cause a delay of the development of the pollen grain and a slight reduction of pollen fertility and plant vigour. They are regarded as belonging to the category of parasitic chromosomes, and are discussed in connection with other similar cases.

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## ABSTRACTS — KURZE MITTEILUNGEN

TORSTEN ROMANUS: Psoriasis in twins.

If a hereditary disease which can appear for the first time at different ages is discordant in monozygous twins, the cause may be that the disease has not had time to show itself in the other twin. When one has to decide whether there is discordance in psoriasis, it is therefore necessary to consider the observation period. In an investigation of 768 post-examined psoriasis cases (ROMANUS, 1945) three-fourths of the men developed the disease before the age of 26 and three-fourths of the women before the age of 19. If, therefore, one reckons with 30 years of age as a limit, it might be said that in most of the cases of psoriasis the disease has appeared at that age.

In the following table are collocated cases of psoriasis in monozygous twins, described in the literature.

Author	Year	Sex	Age, years	Age at onset of psoriasis
<i>Concordance</i>				
SIEMENS	1924	females	32	Since childhood
WEITZ	1925	males	58	No information
CLARK and STIBBENS	1926	»	14	14; 14
V. VERSCHUER	1927	females	12	No information
ZIELER	1930	—	—	» »
VOHWINKEL	1932	males	—	8; 8
WEBER	1934	females	31	22; 26
MAYR	1938	»	c. 34	c. 20; c. 30
KAMPEN	1941	»	14	6; 6
LIEBENAM	1942	»	13	12 $\frac{1}{2}$ ; 13
<i>Discordance</i>				
GLATZEL	1931	males	13	c. 10
DOLLMAN V. OYE	1939	females	10	c. 9
MELSOM	1945	»	18	8
V. VERSCHUER	1927	males	50	No information
GLATZEL	1931	»	38	c. 28
»	»	females	33	No information
ROMANUS	1945	»	31	4
»	the present case	»	43	9

Of the cases in question 10 pairs of monozygous twins were concordant in regard to psoriasis, whereas 4 pairs (with the present case 5) were discordant. Further, discordance has been found for 3 pairs of monozygous twins, aged respectively 10, 13 and 18 years. In these cases, the observation period consequently is rather short.

In the present case (see pedigree) it is a question of two monozygous twin sisters (proband = P), born in 1902. At the time of the examination

they were 43 years of age. They bear a strong likeness which has often led to their being mistaken for each other. Their relatives and friends have mainly been able to distinguish them because of their different temperament. They were born at home and therefore it has not been possible to obtain any information as to the egg membranes.

In the last-born twin (III P) efflorescences of psoriasis appeared on the scalp and the elbows at the age of 9 years. Later on she had disseminated psoriasis punctata on the trunk and the upper extremities. No seasonal variations. She has never been without symptoms. No pregnancy. At the examination in February, 1946, she had a small number of round efflorescences on the scalp and the elbows, varying from  $\frac{1}{2}$  to 1 cm. in diameter.

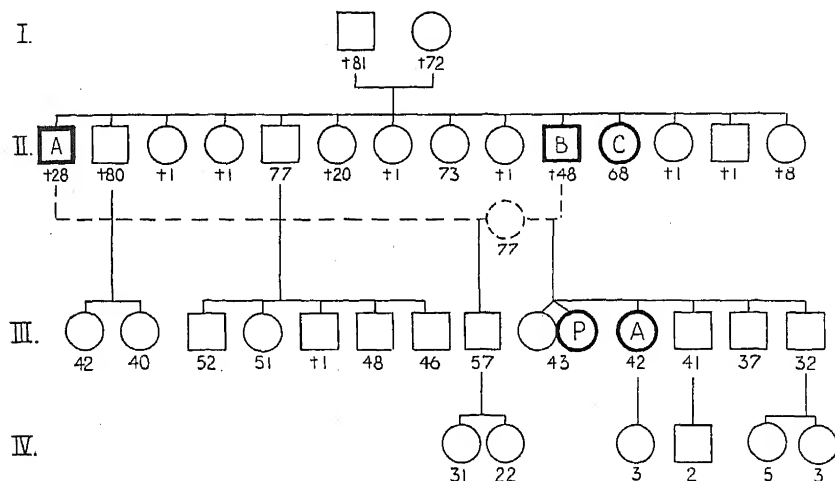


Fig. 1. Pedigree. — Square = man. Circle = woman. A, B, C and P = person affected by psoriasis.  $\wedge$  = twins.

The twin sister has never had any skin affections and did not show any at the examination.

A paternal uncle (II A) who earlier was married to the proband's mother also suffered from psoriasis. According to the record (in 1883) from the Medical Department of the University Hospital at Uppsala where he was treated for psoriasis, the efflorescences appeared at the age of 17 on the left lower leg; later also the scalp, arms and trunk were affected. According to the record, his parents (I) had not had any skin disease; on the other hand, a brother (II B) and a sister (II C) had psoriasis. This sister has in 1946 given the information that her brother, i.e. the paternal uncle mentioned above, after having left the hospital only had few and slight symptoms of psoriasis until his death at the age of 28. It is stated that his son and two grandsons have never had any symptoms of psoriasis.

The proband's father (II B), it is reported, had psoriasis already as a child but only on elbows and legs. He emigrated to the U. S. A. in 1912, which is probably the reason there is no further information about the course of the disease. He died at the age of 48 years.

A paternal aunt (II C) of the proband, who has been examined by the author, has suffered from psoriasis from her childhood but only to a slight degree. She has had circinated eruptions on the trunk, and efflorescences on the elbows; the last 4 years, however, she has only had a few efflorescences on the right elbow, which was the case also at the examination in February, 1946. She has never been free from symptoms.

One of the proband's sisters (III A) has had psoriasis since she was 5 years old. At this age large confluent efflorescences appeared on the lower legs. Later on she had eruptions on the scalp, the trunk and the extremities. At the examination in March, 1946, she had efflorescences on the scalp, and scattered efflorescences on the trunk, elbows and legs. No seasonal variations. Improvement during pregnancy at 39 years of age. She has never been free from symptoms. She has a daughter, 3 years old, who up to the present has not been affected by psoriasis.

We have here a case of psoriasis where the disease has affected only one of two monozygous twin sisters. The cause of the discordance could hardly be too short an observation period, since the twin who suffers from psoriasis got her disease already as a child (9 years of age) and the age of the twins now is 43 years. It is scarcely probable that the twin who up to now has been free of symptoms should be affected by psoriasis later, although this possibility cannot be quite excluded.

There is, however, one more possibility of explaining discordance in monozygous twins. The character may be a genotypic asymmetry (DAHLBERG, 1926 and 1943). From this point of view the localization of the psoriasis-efflorescences is of interest. A paternal uncle had psoriasis only on the left lower leg and, later, on the trunk and arms. A paternal aunt had efflorescences only on the upper part of the body (trunk and arms) and during the last 4 years only on the right elbow. Then there is good reason to believe that in this case the discordance is genotypically determined, so that the gene has only a one-sided effect. In an investigation of psoriasis with asymmetrical localization the present author (ROMANUS, 1945) found that of 478 cases post-examined by him, 71 (14,9 %) had efflorescences with asymmetrical localization. In 8 of these cases the efflorescences were localized (since at least 20 years ago) only on one side of the body. This circumstance as also the cases of discordant psoriasis in monozygous twins speak in favour of the hypothesis that psoriasis can be a genotypic asymmetry, and are also a support of DAHLBERG's theory of genotypic asymmetries.

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# A CASE OF ASYNDESIS IN *PICEA ABIES*

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## I. INTRODUCTION.

SOME investigations have treated in detail the embryology and fertilization of the Gymnosperms (cf. SCHNARF, 1933). Several have also dealt with the somatic chromosome numbers of these plants (cf. TISCHLER, 1926/1927, 1931, 1935/1936 and 1938, as well as A. and D. LÖVE, 1942), but few have been published respecting their chromosome behaviour at meiosis. The chromosome numbers of the Gymnosperms, in contrast to the condition in the Angiosperms, are remarkably constant even between families and differentiated genera. Polyploidy seems to occur very sparingly.

Most of the Gymnosperms have twelve chromosome pairs, or  $2n=24$ . K. and H. J. SAX (1933) were able to determine the chromosome number in the endosperm, which in the Gymnosperms is haploid, for 53 species distributed over 16 genera among the Conifers. The now existing genera of coniferous trees number not fully 50. The investigators just cited found the basic number  $n=12$  in the majority of the Conifers. Species with deviating chromosome numbers are *Cryptomeria japonica*, *Taxodium distichum*, *Taivania cryptomerioides*, *Thuja occidentalis*, *T. orientalis*, *T. plicata*, *T. Standishii*, *Juniperus communis*, *J. virginiana*, *J. rigida*, and *Chamaecyparis Lawsoniana* with the basic number  $n=11$  or  $2n=22$ , and *Pseudotsuga taxifolia* and the *Agathis* species (FLORY, 1936) with the basic number  $n=13$ . For *Podocarpus* FLORY (1936) gives the following haploid numbers: *P. falcatus*,  $+12$ , *P. macrophyllus*,  $+19(-20)$ , *P. neriifolius*, about  $+19$ , *P. andinus*, about  $+20$ , and for *P. chinensis*,  $+20$ . According to TAHARA (1937), the haploid chromosome number of *Sciadopitys verticillata* is  $n=10$ . *Pseudolarix* and *Juniperus chinensis Pfitzeriana* are tetraploid (K. and H. J. SAX, l. c.) with  $2n=44$ . *Sequoia sempervirens* also seems to be polyploid, although no exact chromosome number has been determined. DARK (1932) found about 50 chromosomes in the root-tips of *S. sempervirens* and considers the species to be tetraploid, while K. and H. J. SAX (l. c.) approximately estimated the total chromosome number for the

same species at more than 40. BUCHHOLZ (1939 a and b) found the haploid number  $n=11$  in the sex-cells of *Sequoia gigantea* and the corresponding number  $n=22$  in *S. sempervirens*. The last-mentioned number, however, is given as only approximate. Finally, JENSEN and LEVAN (1941) determined the diploid chromosome number in root-tips of *S. gigantea* at  $2n=22$ . After colchicine treatment of germinating seeds of *S. gigantea* they obtained tetraploid plants having the chromosome number  $2n=44$ .

Among other Gymnosperms polyploidy has only been demonstrated in *Welwitschia* and *Ephedra*. *Welwitschia mirabilis* (FLORIN, 1932) has the chromosome number  $2n=42$  and among *Ephedra* plants *E. nebrodensis* var. *Villarsii*, *E. fragilis* var. *campylopoda* (GEITLER, 1929), *E. americana* and *E. equisetina* (FLORIN, 1932) have  $2n=14$ , while *E. distachya* is tetraploid with  $2n=28$  (FLORIN, 1932). In *Picea* the chromosome number is stated by MIYAKE (1903) to be  $2n=24$ , which has since been verified by K. and H. J. SAX (l. c.).

The present work chiefly deals with the course of meiosis in an asyndetic spruce as compared with that in normal spruces. Some investigations of pollen fertility and seed-setting after selfing and crossing in comparison with the seed-setting after free flowering as well as studies of the progeny raised from different spruces after free flowering will be published in Svensk Papperstidning, 1947. Asyndetic individuals do not seem to have been met with earlier within the Gymnosperms.

## II. MATERIAL AND TECHNICAL METHODS.

### 1. MATERIAL.

At the Brunsberg branch station in Värmland of the Association for Forest Tree Breeding cones were collected during the autumn of 1942 and the spring of 1943, shortly after the free flowering period, from a large number of selected phenotypical élite trees of *Picea Abies* for estimation of the progeny. Sample trees of this type for selective breeding were taken at different levels of altitude and latitude. On the lands of the Billerud Company in the parish of Gunnarskog, West Värmland, cones from 1600 trees were collected in the winter of 1942—1943. During the work of seed extraction and cleaning of these cones it was found that one of the sample trees (spruce No. 181, Gröttvål)

had produced seeds belonging to a different order of size (Fig. 1). All the other trees sampled showed a good production of quite normal seeds. From 24 examined cones of spruce 181 there were obtained 7692 small seeds and 156 or 1,99 % of approximately normal size. Among 200 small seeds that were cut open 196 or 98 % were empty or had not developed any endosperm.

The small seeds were repeatedly placed for germination without a single seed being able to germinate. Among seeds of a *normal size-order*, 17 out of 100 or 17 % sprouted in the germinator at  $+30^{\circ}\text{C}$ .

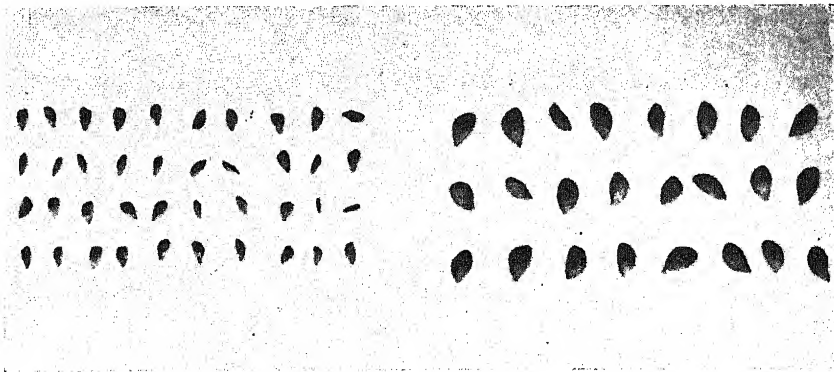


Fig. 1. Seeds of two different orders of size in the asyndetic spruce. —  $\times$  ca. 1,5.

The 1000-grain weight for small and normally sized seeds from spruce 181 and for the average of the other selected trees within the stand is listed below:

*1000-grain weight in grams, year 1943.*

Abnormally small seeds from No. 181 .....	0,88
Normal-sized               »       »       »       » .....	2,75
Mean size of seeds from other trees .....	3,46

Seed from No. 181 was sown in boxes in the spring of 1943, and the resultant seedlings were potted for root fixation. There was a great loss of plants in the boxes, about 30 % of the plants dying. Of 78 surviving offspring, about one-third were characterized by very feeble growth with short needles, and 16 died during cultivation in pots. In some of these plants it was possible to determine the somatic chromosome number.

The mother tree, No. 181, did not flower in the springs of 1943 and 1944. In the autumn of 1944, however, numerous male buds were put forth. In the middle of March, 1945, branches and male flowers were placed in greenhouses for forcing. They were put into glass jars containing water. Male inflorescences were irradiated with two 500 watt lamps every afternoon for four hours. After about 48 hours' irradiation it could be determined by fixation in acetic acid carmine that meiosis had commenced in the buds. For comparison branch and bud material was taken from six sample trees known to have normal seed setting.

In Värmland the meiotic division in spruce started in nature about April 13 in 1945. A large number of staminate inflorescences were then fixed of No. 181 as well as of 20 supposed normal spruce trees. These 20 trees also included the six previously mentioned spruces. The sample trees were derived from the following six localities: Nos. 108, 110, 112, 115, 125 and 140 from Brunsberg, Nos. 166, 220 and 221 from Gröttvål, Gunnarskog, Nos. 4, 17 and 121 from Fredros, Gunnarskog, Nos. 1, 16, 19, and 21 from Vägsjöfors, No. 1 from Lekvattnet, and Nos. 101, 102 and 103 from Höljes in northwestern Värmland. Buds from all the trees were fixed while meiosis was going on in nature. No disturbance whatever in the course of meiosis seems to have been occasioned through bud-forcing under irradiation in greenhouses at  $+12$  to  $+16^{\circ}$  C., buds from the six controls having shown the same normal course of division as buds taken direct from the control trees. On being forced in greenhouses the buds of spruce 181 exhibited the same disturbances at meiosis as when fixed direct in nature.

## 2. CYTOLOGICAL METHODS.

For root fixations use was made of MÜNTZING's modification of NAVASHIN's solution (MÜNTZING, 1932) as well as of LEVITSKY's solution (1931): 8 parts of 1 % chromic acid and 2 parts of 10 % formalin mixed immediately before use. Before staining, objects fixed in LEVITSKY's solution 8:2 were allowed to stand in a mordant of 1 % chromic acid for 12 to 24 hours. Both methods seem to be fully practicable and reliable for root fixations of spruce. At root fixing of offspring from No. 181 the jars with plants were placed on ice overnight for cooling. After being embedded in paraffin the root-tips were cut into 14—17  $\mu$  thick slices and stained with gentian violet.

For studies of meiotic divisions very beautiful stainings were obtained in acetic orcein by the method described by DARLINGTON and LA COUR (1942) as well as in aceto-carmine (K. and H. J. SAX, 1933, and ÖSTERGREN, 1942).

The best permanent preparations of meiotic divisions were obtained by means of the smear method. After smearing, the object glasses were changed over as quickly as possible to MÜNTZING's modification of NAVASHIN's fixative. Smear preparations

were then stained in gentian violet as well as in fuchsine-sulphurous acid (cf. DARLINGTON and LA COUR, 1942) and mounted in Canada balsam. After the smearing the object glasses ought to lie about 30—60 minutes or more in the above-mentioned fixative.

Pollen and stomatal size were determined in aceto-carmin + glycerine (1:1).

The drawings were made with the aid of Abbe's camera lucida and the micro-photographs were taken with a Zeiss spherolux camera.

To enable crosses to be made for breeding purposes between selected elite stocks in nature the Association for Forest Tree Breeding erected specially designed crossing-stages round a number of trees that had already been judged as regards progeny or were presumed to be good genotypes (cf. SYLVÉN, 1943). A crossing-stage of this kind was put up in April, 1945, round the asyndetic spruce 181 at Gröttvål, Gunnarskog (Fig. 2). With the aid of this scaffolding it was possible to take advantage to the flowering time of the tree to self a large number of female cones as well as to effect crosses with other spruces.

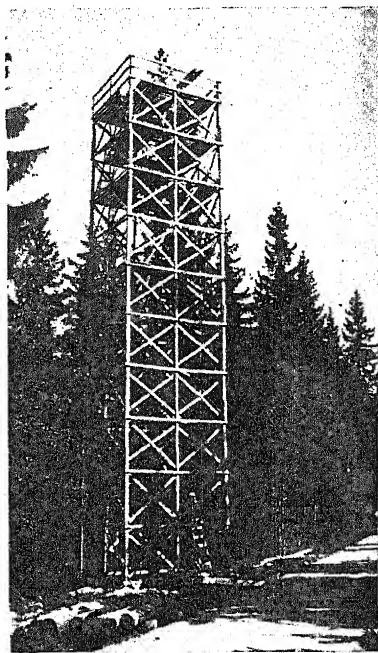


Fig. 2. Scaffolding round spruce No. 181 for crossing purposes.

### III. NORMAL MEIOSIS.

As previously mentioned, for comparison with meiosis in the asyndetic spruce male inflorescences were fixed from 20 spruce trees that presumably possessed a normal meiotic division. Three of these spruces show disturbed meiotic division. This is described later in this work. The other 17 trees have a completely regular meiosis.

During leptotene and zygotene the chromosomes are much elongated and lie scattered at random inside the nuclear membrane. In leptotene the chromosome threads therefore form a reticular bunch, the individual threads of which are usually difficult to distinguish along their whole length.

It is clearly to be seen that the chromosome threads are of granular structure. At pachytene the threads are powerfully reduced in number and are paired. After parallel conjugation a relatively powerful contraction takes place of the chromosomes.



Fig. 3 a. Diplotene in normal spruce. —  
 $\times$  ca. 900.

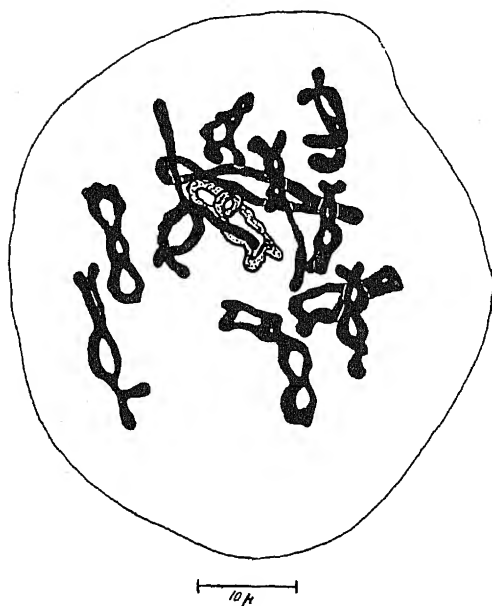


Fig. 3 b. Diplotene in normal spruce. —  
 $\times$  ca. 1300.

During pachytene the chromosomes are distinctly paired, chromomere for chromomere. The chromosomes then increase in thickness, which first manifests itself by a swelling of the chromomeres. A contraction of the chromosomes occurs later in the middle of diplotene. The chromosomes separate more and more clearly from one another along their whole length, except at certain contact-points, as will be seen in Fig. 3 a. In Fig. 3 b the number of chiasmata are represented for each of the 12 bivalents. The mean number of chiasmata per bivalent is at this point of time  $2,81 \pm 0,04$ , as seen in Table 1. Most of the chiasmata are interstitial and persist as such until early metaphase I. Any terminalization of chiasmata is, as we shall see later, either very indistinct or, probably, non-existent.

In the Angiosperms the nucleolus is generally dissolved during the end of the diakinetik stage (DARLINGTON, 1937). The occurrence of the nucleolus during the greater portion of diakinesis, as well as its gradual disappearance during late diakinesis, may therefore be denoted as very characteristic of this stage in

TABLE 1.

Tree No.	No. of cells examined	Division stage	Distribution of bivalents with following number of chiasmata					Mean chiasmata per bivalent
			1	2	3	4	5	
112	30	Diplotene		149	150	43	18	$2,81 \pm 0,04$
112	30	Diakinesis		151	148	45	16	$2,79 \pm 0,04$
112	24	Metaphase I	3	118	115	43	9	$2,78 \pm 0,04$
101	30	"	9	158	129	53	11	$2,72 \pm 0,05$
220	30	"	6	153	136	54	11	$2,75 \pm 0,04$
121	30	"	2	155	141	52	10	$2,76 \pm 0,05$
4	24	"	2	118	116	45	7	$2,78 \pm 0,05$
16	20	"	5	102	99	28	6	$2,70 \pm 0,05$
19	20	"	6	96	103	27	8	$2,73 \pm 0,05$

the Angiosperms. In *Picea Abies*, on the other hand, the dissolution of the nucleolus seems to take place considerably earlier and as a rule is going on at the beginning of diplotene. At the end of diplotene and during the diakinetik stage cells with a nucleolus are only rarely found.

All chiasmata remain unchanged in diakinesis. Among other signs showing this is the chiasma frequency in 30 analysed cells of spruce 112. In these cells the mean number of chiasmata per bivalent was found to be  $2,79 \pm 0,04$  during diakinesis. The same spruce showed a mean number of chiasmata per bivalent for the same number of cells during diplotene as was earlier observed, viz.  $2,81 \pm 0,04$ . The difference between these means is not statistically significant.

In Fig. 4 a cell of spruce 112 is seen during diakinesis. The chromosomes are much contracted and the bivalents are of about the same order of magnitude as during metaphase I. Here the number of chiasmata per bivalent is 2,83. This mean varies somewhat from cell to cell. The mean number of chiasmata per bivalent as between cells has varied during diakinesis between 2,67 and 3,0. The intra-cell variation is definitely greater and ranges from 2,0 to 5,0 chiasmata per bivalent. Solitary bivalents with one chiasma have been found only in metaphase I (cf. Table 1). Bivalents with more than 5 chiasmata have not been observed. As is evident from most of the illustrations, the bivalents vary in length. All the chromosomes have median or submedian centromeres (cf. also K. and H. J. SAX, 1933).

Metaphase I is illustrated by Figs. 5 and 6. Sometimes it occurs that a bivalent reaches the equatorial plane too late to range itself among the others. Such a late-comer will often be excluded from the common divisional plane of the other bivalents. This is probably due

to there being insufficient space on the metaphase plate. Under such circumstances this bivalent must divide alongside the equatorial plane of the cell.

The relative sizes of the bivalents during metaphase I and the number of chiasmata per bivalent and their positions as well as the positions of the centromeres are illustrated in Figs. 5 and 6. In both figures the bivalents are drawn separately — without taken account



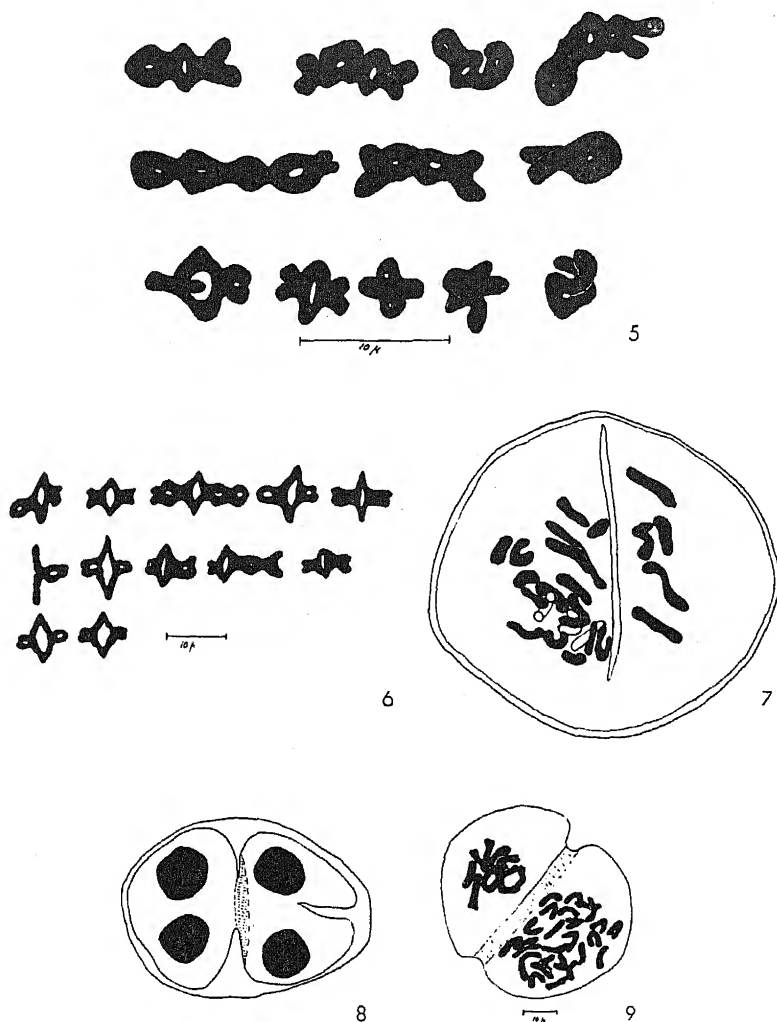
Fig. 4. Diakinesis in normal spruce. —  $\times$  ca. 900.

of their relative positions in the cell. Each figure contains, however, a complete set of bivalents.

Figures showing the chiasma frequency during metaphase I are given in Table 1. The number of chiasmata per bivalent ranges from  $2,70 \pm 0,05$  to  $2,78 \pm 0,05$ . As will be seen, terminalization of chiasmata does not occur in proportion to the number of chiasmata per bivalent during diplotene and diakinesis. From the table it is also evident that the variation between trees in respect of the mean number of chiasmata per bivalent is not statistically significant.

According to DARLINGTON (1937), a minimum terminalization may partially depend upon the size of the chromosomes, for in *Stenobothrus* amongst others he

observed that the small chromosomes invariably terminalize completely whereas the process is slow and incomplete in the case of the large chromosomes. On the other hand, in other species, e.g. of *Allium*, no such correlation seems to exist between terminalization and chromosome size. From a comparison of different species



Figs. 5—6. Bivalents drawn separately during metaphase I in normal spruce. — Fig. 7. Early metaphase II. Division wall being laid down centrifugally during metaphase II in the asyndetic spruce. — Fig. 8. Laying down of division walls in microsporocytes in normal spruce. — Fig. 9. A cell-plate has developed and tendency to »Furchung» or centripetal wall formation is already present during metaphase II in the asyndetic spruce. — Fig. 5,  $\times$  ca. 2100. Figs. 6—7,  $\times$  ca. 900. Figs. 8—9,  $\times$  ca. 450.

DARLINGTON therefore infers that other factors, such as longitudinal contraction and repulsion of the centromeres, exercise a great influence on terminalization quite independently of chromosome size. MATHER (1940) has advanced very strong reasons for assuming that chiasmata always strive for definite positions and that no visible change in position occurs after chiasma formation has taken place, except in those cases in which complete terminalization occurs. He therefore distinguishes between chiasmata that terminalize and such as remain interstitial. He says: »Thus, in general, terminalization is an all or none process».

First anaphase and the succeeding stages of division in the spruce proceed as normally and schematically as the prophase and first metaphase divisions. Sometimes there persist for a time terminal connections or chromatin bridges between one or a pair of chromatids after the others have separated. Fragments or chromosome bridges with fragments have never been observed. When the chromosomes have reached the poles two daughter nuclei invested by nuclear membranes are formed in a normal manner. After that, the daughter nuclei enter into a resting stage, during which the chromosomes are difficult to distinguish and in course of time assume an appearance which to some extent resembles that of the mother-nucleus before division. In many daughter-nuclei during telophase I there appear 3—10 small globular bodies. Probably, extra nucleoli are concerned here similar to those described in *Pisum* by HÅKANSSON and LEVAN (1942). These bodies appear during telophase I in most of the pollen mother-cells, though in varying number. During early interkinesis most of them probably coalesce. By the end of interkinesis the number has often been reduced to two or three nucleoli, which later entirely disappear before second prophase.

No support has been found by me in the spruce for the hypothesis that the interkinetic stage is especially prolonged in time in the Gymnosperms as compared with other meiotic stages. TISCHLER (1943) states with respect to the length of the interkinetic stage: »In der älteren Karyologie wird angegeben, dass in gewissen Pflanzengruppen wie Pteridophyten und Gymnospermen die Neigung zu langen Interkinesen vorherrschen kann. Belege dafür boten *Botrychium*, *Helminthostachys*, *Equisetum*, *Larix*, *Taxodium*, *Pinus*, *Thuja* u. a.». TISCHLER himself, however, considers that external time factors probably have a greater influence than the genetic on the course of interkinesis.

Second division follows the course of an ordinary mitosis. The chromosomes arrange themselves in regular plates and divide lengthwise, which in reality means that only the centromeres divide, since

the chromosomes are already divided into two chromatids. Second anaphase likewise proceeds quite normally and gives rise to four tetrad nuclei. As a rule the tetrads lie in the same plane.

#### IV. MICROSPORE FORMATION IN PICEA ABIES.

In *Picea Abies* the tetrad formation follows the simultaneous scheme as in *Pinus silvestris* (VOGEL, 1936). In the asyndetic spruce the simultaneous tetrad division is also the most commonly occurring, although the succedaneous course of division has also been observed in a number of cells. The literature contains very divergent statements concerning tetrad division in the Gymnosperms. A collocation of the microsporogenesis in the Gymnosperms has been made by SCHNARF (1933, pp. 6—10). Data relating to the production of microspores within Pinaceae are mainly confined to *Pinus* and *Larix* and are lacking as regards *Picea*.

*Pinus* and *Larix* represent the simultaneous type (NEMEC, 1910; DÉWISÉ, 1922; PROSINA, 1928; STRASBURGER, 1895; FERGUSON, 1904; JURANYI, 1882). Other, rather deviating particulars have been given by COULTER and CHAMBERLAIN (1917) respecting *Pinus laricio*, the division of which may take place both simultaneously and succedaneously. According to COULTER and CHAMBERLAIN, the last-mentioned division is the commonest. In *Larix europaea* var. *pendula* the microspore formation is also reported to be able to occur either according to the simultaneous or the succedaneous scheme.

As a rule the tetrad division proceeds rapidly in *Picea Abies*, as does also wall formation between the tetrad nuclei.

Following the heterotypic division certain thickenings in the plasm are now and then seen in telophase I as well as traces of a cell-plate, though this plate disappears and is not laid down until anaphase II or after the tetrad nuclei have been formed. In spruces having normal meiosis the pollen mother-cells follow the simultaneous scheme. In the asyndetic spruce dividing walls are formed in some cases during metaphase II. Of 633 cells in metaphase II, 29 or 4.6 % had developed dividing walls. No compartment walls are formed immediately after the heterotypic division; however, they are laid down during metaphase II. Fig. 7 shows a cell during early metaphase II, where the dividing walls are under construction. The compartment walls growing out during metaphase II seem as a rule to arise by a new formation of cell-plates in the middle of the cell, these growing out centrifugally towards the periphery of the cell (Fig. 7).

Generally the cell-plate is thickest in the middle of the cell and therefore has the appearance of a very small and pointed nuclear spindle. However, the incomparably commonest way in which new dividing walls seem to be formed is by the joint action of cell-plates and cell-constrictions or so-called »Furchung» (Fig. 8). A similar wall formation in microspore-cells appears to have been found by MANN (1924) in *Ginkgo biloba*. Frequently the first cell is divided into two »dyad cells», which in their turn are divided by cell-plate formation and »Furchung». Sometimes no complete cell-plate is seen at this division, the dividing walls appearing to arise solely as a result of »Furchung». *It is however most probable that a conjoint action of cell-plates and cell-constriction is always involved in the construction of the dividing wall.* In Fig. 9 a cell of spruce 181 is represented during metaphase II, where a cell-plate has been formed and a tendency to cell-constriction or centripetal wall formation exists.

With regard to the laying down of the separating walls of the microspore cells in *Pinus silvestris*, VOGEL (1936) states among other things: »Während der zweiten Teilung wurde die ganze Zelle mit zusammenhängenden, unregelmässig verlaufenden Plasmafäden erfüllt. Hieraus entstanden zarte Wände, die die Mikrosporen entstehen liessen und voneinander abschlossen . . . Bald verdickten sich diese Wände und vereinigten sich mit der inneren Wand der Sporenmutterzelle. Es erfolgt weitere Verdickung der äusseren Wand, mit der eine Verstärkung der die Tochterzellen trennenden Wandung parallel geht». VOGEL's exposition gives the impression that, to begin with, the thin dividing walls hang freely in the cells, which is probably not the case.

## V. COURSE OF MEIOSIS IN THE ASYNDETTIC SPRUCE No. 181.

### 1. PROPHASE.

During early prophase in spruce 181 no major deviations are found from the course of division in a normal spruce. Leptotene and zygotene seem to proceed normally. Chromosome pairing in early pachytene is approximately as complete in the asyndetic spruce (Fig. 10) as in any spruce with normal reduction division. Even during late pachytene and early diplotene there is a very distinct difference between the asyndetic type and the normal. All previously formed spirals between paired chromosomes within the bivalents seem to regress and the

chromosome pairs gradually fall apart and numerous univalents begin to appear. It is therefore clear that on the whole there has been a failure in the formation of real chiasmata. At the point when the chromosome contraction becomes noticeable during diplotene earlier contact-points within the chromosome pairs seem to vanish. Signs of chiasmata or crossing-over points between paired chromosomes are observed in solitary cases during diplotene, but it is impossible to

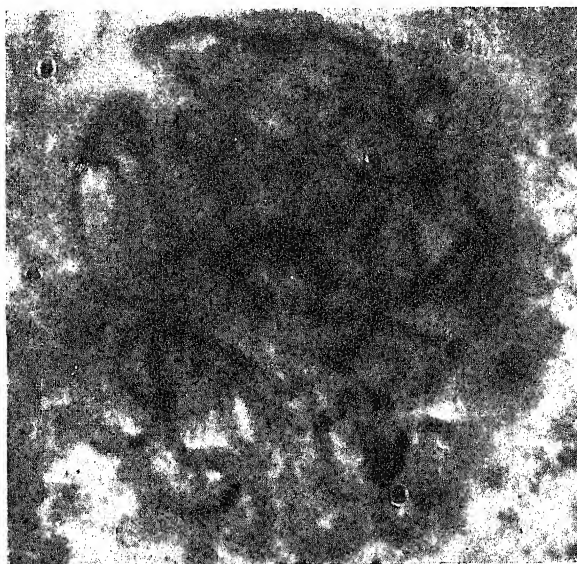


Fig. 10. Early pachytene in the asyndetic spruce. —  $\times$  ca. 3000.

determine whether in such cases it is a question of real chiasmata or merely contact-points, or relational coiling.

At diakinesis the difference between the asyndetic and normal spruce types is very distinct. Often 24 univalents are observed in every pollen mother-cell of spruce 181 (Fig. 11) or cells with 1—3 bivalents and respectively 22 and 18 univalents. Multivalents or coalesced chromosomes also occur in low frequency. Almost all the bivalents that occur have one chiasma. More rarely ring bivalents or bivalents with two chiasmata are observed. These chiasmata are generally terminal. Diakinesis passes over into metaphase I without any great noticeable changes.

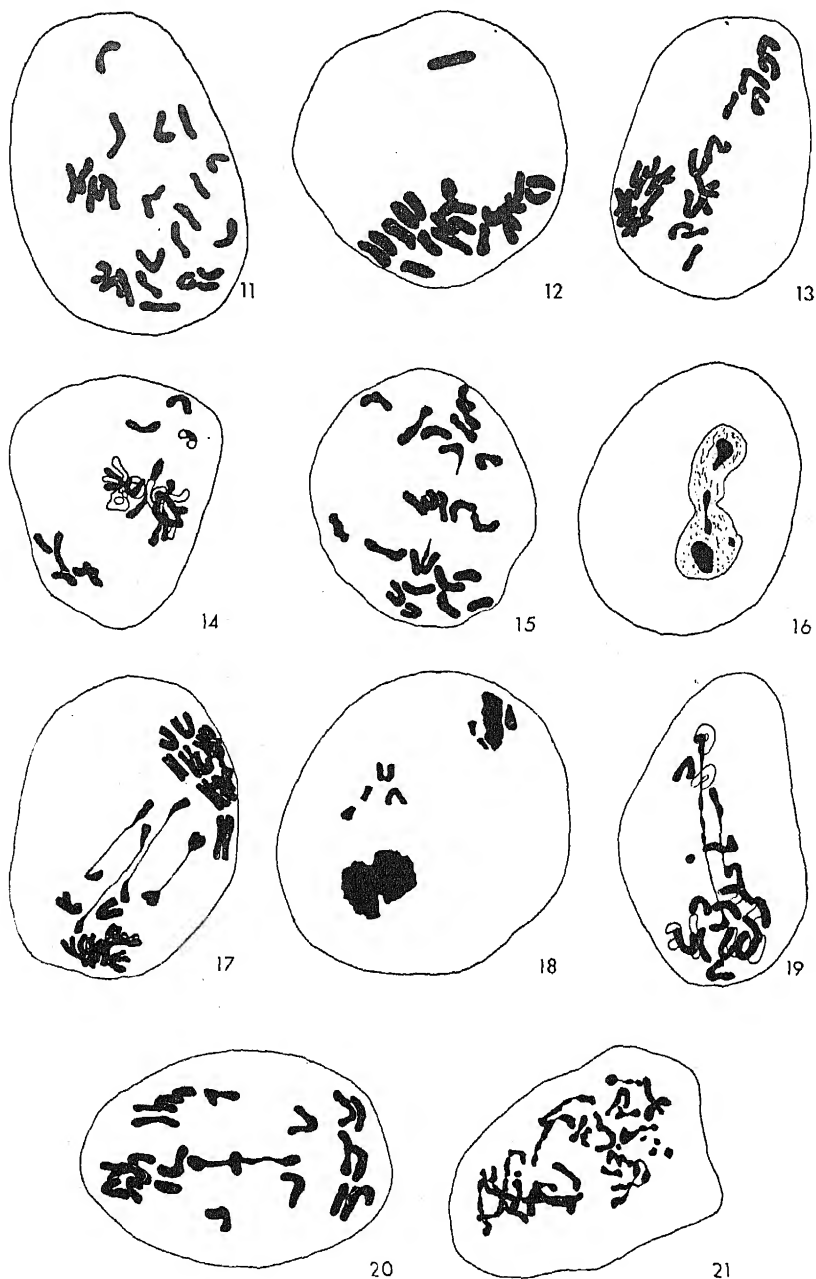


Fig. 11. Diakinesis — early metaphase I in the asyndetic spruce. — Figs. 12–14. Metaphase I. — Fig. 15. Anaphase I. Some univalents remain at the equator after the paired chromosomes of the bivalents have separated. — Fig. 16. Restitution

## 2. FIRST DIVISION.

During early metaphase I the chromosomes in cells with solely or almost solely univalents lie scattered around in the cells. In cells with multivalents, on the other hand, almost all the chromosomes are clustered together. During metaphase I all kinds of transitional forms appear, and the chromosomes are mostly arranged more or less asymmetrically to the equatorial plane of the cell and the longitudinal axis of the spindle.

In the asyndetic spruce the different meiotic stages of the first division are not so sharply differentiated as in normal meiosis. Earlier it was pointed out that the transition from diakinesis to metaphase I is less distinct in the asyndetic spruce. Almost as diffuse is the transition from metaphase I to anaphase I in spruce 181. This is largely due to the fact that in many cells only or almost only univalents occur. The univalents are scattered all over the cell. As a rule, they do not first of all arrange themselves into a nuclear plate in the equatorial plane of the cell but migrate direct to the poles (Fig. 12). Moreover, this separation toward the poles mostly takes place successively. The meiotic divisions in the normal and asyndetic types also differ in respect of time. The first division takes about double as long in the asynaptic spruce. There is however a smaller difference in time between the second divisions of the two types. Out in nature the meiotic division of the normal type goes on for 2—3 days. The corresponding time for spruce 181 is 4—6 days.

A strong influence is naturally exercised on the division intensity by the temperature and light conditions. When male inflorescences of spruce are being forced in a greenhouse, light seems to have a greater effect than heat on the rate of division. In strong sunshine the second division of spruce in nature under a temperature of about  $+15^{\circ}$  C. may go on for two or three hours. During metaphase I one or more bivalents occur in about two-thirds of the total number of pollen mother-cells. More than five bivalents per cell is very rare. With the exception of some rod bivalents with interstitial and median chiasmata all bivalents have terminal chiasmata. The majority of these consist of rod bivalents with one chiasma. In solitary cells multivalents have been

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nucleus. — Fig. 17. Anaphase I with misdivision of univalents. — Fig. 18. Late anaphase I—telophase I with fragments, which have probably arisen through misdivision. — Figs. 19—20. Late metaphase I. — Fig. 19 shows a »bridge» with a fragment. — Fig. 20. Bivalent with interstitial chiasma. — Fig. 21. Late diplotene. Cell with much fragmentation. —  $\times$  ca. 800. (The cell in Fig. 12 was fixed in acetic orcein.)

observed. Among 500 examined pollen mother-cells multivalents occurred in seven or in 1.4 % of the total number of pollen mother-cells. In a number of cells the chromosomes are fased together and cannot therefore be counted. The number of bivalents per cell has been counted in 1164 cells. A division of the cells with respect to the number of rod and ring bivalents per cell and the mean number of chiasmata per bivalent within the different groups is given below.

Number of bivalents per cell	0	1	2	3	4	5	6	7	8	Total number of cells
» » cells with 0—8										
rod bivalents ..	456	226	220	92	40	33	13	3	5	1088
» » cells with rod and ring bivalents			18	16	8	11	3	1	3	60
» » ring bivalents in these cells .....			(18)	(19)	(13)	(22)	(7)	(3)	(13)	—
» » cells with solely ring bivalents ..		14	2							16
Mean number of chiasmata per bivalent .....		1,06	1,05	1,06	1,07	1,10	1,07	1,11	1,20	
Number of cells	456	240	240	108	48	44	16	4	8	1164

From the table it will be seen that the number of chiasmata per bivalent increases on account of the fact that the percentage of ring bivalents per cell rises with increased number of bivalents per cell. The mean number of chiasmata per bivalent in the cells examined is 1,07. Real chiasmata thus appear to be formed in the asyndetic spruce, although the bivalent frequency is very low. In the examined pollen mother-cells bivalent formation was entirely absent in 39 %. In other cells examined there were 1644 bivalents out of 8496 possible ones. Expressed in percentage, the bivalent formation in cells with one or more bivalents will be 19,35 % of the number of possible bivalents. Of this frequency 113 out of 1644 bivalents, or 6,87 %, consist of ring bivalents. In all examined pollen mother-cells from No. 181 the frequency of bivalents is only 11,77 % of the number of possible bivalents, or comparable with the bivalent frequency in spruce trees having normal meiosis. A division of this frequency into rod and ring bivalents gives 10,96 % rod bivalents and 0,81 % ring bivalents. Almost all ring bivalents have terminal chiasmata, the number possessing interstitial ones being insignificant. A single ring bivalent was observed with three chiasmata; all the rest had two.

Besides ring bivalents, metaphase I and anaphase I also exhibit ring-shaped univalents that have terminally attached chromosome-ends

and thus form a closed ring. These ring univalents were observed in 37.2 % of the number of pollen mother-cells counted. The idea readily suggests itself that these ring univalents may be iso-chromosomes which have arisen through misdivision in one of the parental trees of the asyndetic spruce. Such iso-chromosomes have double segments and are therefore able to pair. A summary of works on iso-chromosomes is given by MÜNTZING (1944, pp. 245—246; cf. also 1945). In No. 181 some cells contain one to three ring univalents, which makes it highly improbable that iso-chromosomes are concerned in this case. Therefore, a more likely explanation is that the ring univalents present have merely stuck together and that the attachment between the chromosome-ends is of a heterochromic nature. The frequency of ring univalents per cell is as follows:

No. of ring univalents per cell	0	1	2	3	4	Total number of cells
No. of pollen mother-cells . . . . .	302	141	33	5	0	481

The second division is also marked by the occurrence of solitary ring univalents, which makes it still more improbable that this is a case of iso-chromosomes.

During metaphase I the relative positions of the chromosomes in the cells exhibit highly variable metaphase pictures. The chromosomes may either be evenly distributed (Fig. 11), heaped in the middle of the cell or at the cell-wall (Fig. 12), arranged in metaphase plates, which may be more or less displaced from the equatorial plane of the cell, or strewn at random round the nuclear spindle (Fig. 13). The chromosome configuration in Fig. 13 has either been caused by a tripolar or a crescent-shaped spindle. This latter alternative would seem to be the more probable in this case. Tripolar spindles occur in low frequency in normal spruces, while abnormally stretched and semilunary curved spindles are only met with in the asyndetic type (cf. also DARLINGTON, 1937, p. 415). These different metaphase pictures are a direct consequence of the *behaviour of the univalents during metaphase I*. When no bivalents occur in the cells, the univalents mostly lie strewn at random round the cell or along the nuclear spindle. The univalents do not as a rule arrange themselves in a metaphase plate but preferably migrate direct to the poles. Their distribution on the two poles certainly seems to take place entirely at random (Fig. 12), although there is poor agreement with the theoretically expected distribution. When the chromosome groups are well separated, two interkinetic nuclei arise.

Occasionally some univalents may remain in the equatorial plane of the cell. These afterwards migrate either to one of the poles to enter into the two daughter-nuclei or to remain there and form an extra interkinesis nucleus. It also occurs that the univalents retarded at the equator join the two other chromosome groups and give rise to one or more restitution nuclei. Restitution nuclei occur however in very low frequency in spruce 181.

In the presence of bivalents the majority of the univalents also seem to arrange themselves in a metaphase plate. Together with the bivalents they form a common plate at the equator (Fig. 14) or at the side of the latter. Univalents associated with real bivalents seem to have a tendency to behave as »bivalents» and to form a common plate with them. Those univalents which are not included in the metaphase plate generally remain in their original positions during division of the bivalents. Most frequently, however, one or more univalents are observed at or near the poles at the time the bivalents divide. These univalents must either have reached the poles before formation of the metaphase plate or have passed to the poles after the rest of the chromosomes had arranged themselves in a plate. When the paired members of the bivalents have separated and commenced their migration to the poles, the univalents also begin to shift towards one of the poles. During this anaphase migration other univalents scattered around in the cell also make their way to one of the two poles.

Only in a few pollen mother-cells do one or more univalents remain in the equatorial plane of the cell and divide after the paired chromosomes of the bivalents have separated (Fig. 15). After that, the two univalent halves or the chromatids either pass each to its pole and are included in the daughter-nuclei or remain at the equator and are marked off from the anaphase nuclei in small micronuclei. These split univalents may serve as connecting links between the anaphase nuclei and give rise to restitution nuclei (Fig. 16) if the separation of chromatids or possibly of only longitudinally divided univalents with undivided centromere takes place before the daughter-nuclei have invested themselves in a cell-membrane.

Univalent splitting seems to be rare in the pollen mother-cells of absolutely asynaptic Angiosperms with one or more bivalents. Only among hybrids between *Solanum tuberosum*  $\times$  *S. curtilobum* (LAMM, 1941) and certain inbred plants with absolute asynapsis in *Alopecurus myosuroides* (JOHNSSON, 1944) have split univalents been found. On the other hand, such are common at anaphase I in plants with partial or rather weak asynapsis. This is the case in e. g. *Hordeum* (EKSTRAND, 1932),

*Crepis* (RICHARDSON, 1935), *Pisum* (KOLLER, 1938), *Populus* (JOHNSON, 1940), and *Secale* (PRAKKEN, 1943). In *Picea Abies* split univalents occur in anaphase I not only in cells with one or more bivalents, which may be regarded as cells with partial asynapsis, but also and most frequently in cells that have no bivalents. The centromeres divide in anaphase I after the univalents have first oriented themselves in a regular metaphase plate.

During late anaphase I the univalents distributed to the poles also divide lengthwise with the exception of the centromeres. The chromatids seem to be distributed both regularly and irregularly to the poles — undivided univalents occasionally pass at the same time as divided univalents to both the poles. A similar case has already been described by LAMM (1941) in sterile hybrids from the cross *Solanum tuberosum* × *S. curtilobum*. Bivalents also seem sometimes to pass undivided to the poles. This is particularly the case when only one bivalent occurs per cell. Such cases of non-disjunction, however, occur very rarely. Figs. 17—18 illustrate indications to misdivision, which probably occurs in solitary cells. Fig. 17 shows some lagging univalents while division is in progress. Their centromere regions are filamentously drawn out into bridges, and the centromeres do not appear to be able to divide. The fragments appearing in Fig. 18 are probably products of misdivision. These four fragments have presumably arisen through the transverse division of the univalents. As in haploid rye (LEVAN, 1942), telocentric fragment-chromosomes have been observed in a number of cells during the second division.

Misdivision has been specially discussed and described by DARLINGTON (1939, 1940) in *Fritillaria kamschatkensis*. Further cases have since been found in several materials, e. g., in *Godetia Whitneyi* (HÅKANSSON, 1940 a and b), *Secale* (LEVAN, 1942; PRAKKEN, 1943; MÜNTZING, 1944), and *Beta* (LEVAN, 1945). Even before 1939 cytological observations that can be interpreted as misdivision were made by several workers (cf. MÜNTZING, 1944).

As already mentioned, in a number of pollen mother-cells one or more split univalents make their appearance. The number of cells with split univalents and the number of split univalents per cell in 1164 cells are summarized below:

No. of split univalents per cell	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
No. of cells containing split univalents	10	6	3	5	2	1	0	1	1	0	3	2	0	0	4	2	0	0	2	1	0	2	1	2

Among 1164 pollen mother-cells split univalents were observed in 48 cells or in 4.1 % of the number of cells. In two cells all the uni-

valents were divided. The longitudinal division and the division of the centromeres in the univalents take place during anaphase I. Sometimes the division is incomplete in so far as the centromeres of a number of univalents remain undivided and only the chromatids separate. In the above collocation of split univalents, therefore, only such as possess divided centromeres have been included.

Besides the previously described anomalies in the meiotic division of the asyndetic spruce there occur considerably drawn-out bivalents with fragments — resembling inversion bridges with fragments — together with stickiness in a number of pollen mother-cells. Among the 1164 cells examined band-like drawn-out terminal or interstitial connections between some chromosomes have been observed in 161 cases or in 13.83 % of the number of examined and analysable pollen mother-cells. In these cells only one bridge per cell was found as a rule; in but few cases were 2—5 bridges per cell observed. Figs. 19—20 illustrate some bivalents and bridges of this kind. All these bivalents have one chiasma, as will be seen from the illustrations.

Judging from their positions and from the shape of the bivalents, the repulsion between the centromeres of the bivalents would seem to be of fully normal strength. Those parts of the bivalents which are located between the centromeres and chiasmata are mostly drawn out into long threads or chromosome bridges. In 13 of the 161 cells, or in 8.1 % of the number of cells with such bivalents, there are fragments of varying size together with bridges. This suggests the occurrence of inversion bridges, which would have arisen through crossing-over within the inversion coils. Thus, fragments occurring together with chromosome bridges would afford a strong support for the assumption that real inversion bridges are concerned in the thirteen cases just cited.

Arguing against this assumption is the absence of inversion bridges in normal spruces as well as the type of the bridges. There is reason to expect that the number of inversions in the asyndetic spruce is less than in the normal type owing to reduced chiasma formation. The general type of inversion bridge has not been met with in the asyndetic spruce. The fragments found together with the bridges need not necessarily be interpreted in this case as a sign of inversions.

Fragments also occur in a few cells without bridges. These fragments already arise at early diplotene through collapse of the chromosomes, which takes place in solitary cells. Fig. 21 illustrates such a transversal collapse of the chromosomes during late diplotene. In some cells showing much fragmentation there arise at metaphase I chromo-

During anaphase I the univalents in the asyndetic or asynaptic spruce appear to be distributed more or less at random to the two poles. In cells with solely or almost solely univalents there would therefore be reason to expect a completely random distribution of the chromosomes. The 12 : 12 distribution should quite naturally be the most frequently occurring one, while distributions such as 1 : 23, 2 : 22, 3 : 21 or 23 : 1, 22 : 2, 21 : 3, etc. ought to occur very sparingly if the distribution is to be binominal. To determine the chromosome distribution in different pollen mother-cells during anaphase I 186 cells were examined. The results are presented in Table 2. In making the chromosome counts the cell-pole that had been directed towards the north or the west part of the visual field in the microscope was invariably counted and recorded first. By this means the 17 : 7 distribution could be distinguished from the inverse one, 7 : 17, which theoretically ought to occur equally often as the former. This procedure is not necessary unless it is desired to record the distribution of the pollen mother-cells with regard to the chromosome distribution in the same manner as a binominal series.

Distribution												
of chromosomes: 0:24 1:23 2:22 3:21 4:20 5:19 6:18 7:17 8:16 9:15 10:14 11:13												
<hr/>												
No. of pollen												
mother-cells:	1	3	5	3	5	4	7	9	10	12	9	15
Expected												
distribution:	0,00	0,00	0,00	0,02	0,12	0,47	1,49	3,84	8,15	14,50	21,74	27,67
<hr/>												
Distribution												
of chromosomes: 12:12 13:11 14:10 15:9 16:8 17:7 18:6 19:5 20:4 23:1 22:2												
<hr/>												
No. of pollen												
mother-cells:	21	13	9	11	9	10	8	5	3	4	3	
Expected												
distribution:	29,98	27,67	21,74	14,50	8,15	3,85	1,49	0,47	0,12	0,02	0,00	
<hr/>												
Distribution												
of chromosomes: 23:1 24:0												
<hr/>												
No. of pollen												
mother-cells:	4	3										
Expected												
distribution:	0,00	0,00										

Table 2 compares the found distribution of the pollen mother-cells with the binominal distribution  $(p + q)^{24}$ . As the probability that a certain chromosome will pass to a certain pole during anaphase I is  $\frac{1}{2}$ , the two probability values in this case have the value  $\frac{1}{2}$ . If the binominal  $(\frac{1}{2} + \frac{1}{2})^{24}$  is expanded and multiplied by the number of pollen mother-cells, the expected distribution will be obtained. There is undoubtedly a very bad agreement between the found and the expected distribution, and it is beyond all doubt that the two series are not identical. Not even a very powerful increase in the number of pollen mother-cells examined can in this case alter the agreement between the two series. This is most clearly evident from the direct probability values for the different classes obtained by expanding the binominal  $(\frac{1}{2} + \frac{1}{2})^{24}$ . In this expansion we obtain in the numerator the following binominal series: 1; 24; 276; 2.024; 10.626; 42.504; 134.596; 346.104; 735.471; 1.307.504; 1.961.256; 2.496.144; 2.704.156; 2.496.144; 1.961.256; 1.307.504; 735.471; 346.104; 134.596; 42.504; 10.626; 2.024; 276; 24; 1. The common denominator for all terms will in this special case, when  $p$  and  $q$  are equal, then be 16.777.216. From this binominal distribution it is seen that the number of expected pollen mother-cells having, for instance, the 1 : 23 and 23 : 1 chromosome distribution will in each case be 24 out of 16.777.216 possible cases. According to the same distribution, the probability of finding cells with the 0 : 24 or 24 : 0 chromosome distribution is altogether only 2 cases out of 16.777.216.

We see from this that even with a very large number of examined cells the values for the extreme classes in the expected distribution will be 0. Utilizing the  $\chi^2$  method as a criterion for judging whether hypothesis and observation are in agreement with each other, the quotient between the difference of the series for the lowest classes and the expected distribution (0) will consequently be infinitely large. Apart from these distribution classes the observed and expected frequencies are statistically quite different from each other. Only the  $\chi^2$  values for a couple of classes are necessary to give a  $P$  value of 0,001, which is immediately obvious from the two distributions, or perhaps still better from the following arrangement in which classes with in reality the same distribution have been grouped together:

0:24, 1:23,	2:22,	3:21,	4:20,	5:19,	6:18,	7:17,	8:16,	9:15,	10:14,	11:13,	12:12
11	8	7	8	9	15	19	19	23	18	28	21
0	0	0,04	0,24	0,94	2,98	7,68	16,30	29,00	43,48	55,34	29,98

Thus, the distribution of pollen mother-cells with varying or equal

numbers of chromosomes at the poles does not agree with the expected binominal distribution. The chief cause of this seems to be that the chromosomes have a certain tendency to appear in groups. These groups cause a considerable displacement in the expected binominal distribution of the chromosomes, behaving as they often do as one unit and entirely or partially migrating to one of the poles. This is especially the case in non-disjunction, which phenomenon *per se* implies an irregular distribution of the chromosomes. To this comes the fact that univalents plus one or a couple of bivalents will sometimes form a common group that often also behaves as one unit and passes to one or the other of the two poles.

### 3. INTERKINESIS.

During interkinesis more than two interkinetic nuclei frequently arise as a consequence of an irregular first anaphase separation. In several cases three or four nuclei per cell were observed and in one case five interkinetic nuclei among 274 examined cells. Cells with more than five nuclei occur rather sparingly. Still, in one cell 16 interkinesis nuclei could be distinctly observed, which shows that a single chromosome is able to form an extra nucleus. In cells having two nuclei the interkinesis nuclei are often of different size. As was expected, unicellular interkinesis also occurs in a number of cells<sup>1</sup>. Sometimes univalents and fragments in the plasma also appear outside the interkinetic nuclei.

### 4. SECOND DIVISION.

The second meiotic division is fairly normal in most of the cells, i. e. cells which contain solely double chromatids and are devoid of fragments. All other cells with one or more single chromatids, fragments, stickiness, three metaphase groups, or chromatin bridges are marked by a series of irregularities. This last group of cells consists of about 25 % of the number of pollen mother-cells. As a consequence of the irregular distribution of the chromosomes during anaphase I pollen mother-cells containing a highly varying number of chromosomes occur during the second division (Figs. 7, 9 and 22). A relatively good agreement exists between the distribution of univalents observed during metaphase I and early anaphase I and the number of chromosomes in the configurations during metaphase II. This correlation is brought out clearly by the following comparison.

<sup>1</sup> Out of 274 examined cells, restitution nuclei were formed in 24 cases or 8,76 %.

Chromosome distribution among daughter-cells									
No. of pollen mother-cells:	0:24,	1:23,	2:22,	3:21,	4:20,	5:19,	6:18,	7:17,	8:16
In anaphase I .....	4	7	8	7	8	9	15	19	19
In metaphase II .....	15	5	6	8	7	6	12	15	14
No. of pollen mother-cells:	9:15,	10:14,	11:13,	12:12	No. of cells laid down				
In anaphase I .....	23	18	27	21	186				
In metaphase II .....	18	28	36	23	193				

The number of cells having an unreduced chromosome number has increased substantially in comparison with what was the case after distribution of the univalents during metaphase I. This is due to the

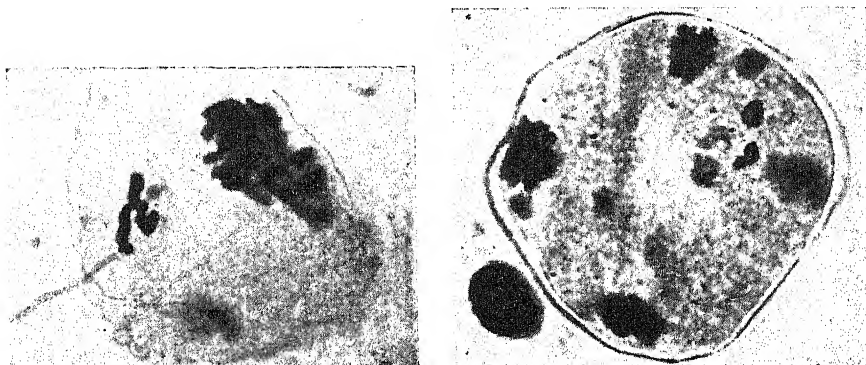


Fig. 22. Metaphase II in the asyndetic spruce. — Fig. 23. Telophase II with single chromatids remaining on the equator. —  $\times$  ca. 800.

appearance of several restitution nuclei in anaphase I on account of the fact that lagging univalents in the equatorial plane and split univalents in certain cells have succeeded in establishing connecting links between the daughter-nuclei, which as a result have been united to one restitution nucleus. Some cells contain single as well as double chromatids. The single chromatids derive from the univalents divided in anaphase I. In metaphase II these chromatids are unable to divide and therefore remain on the equator during anaphase II (Fig. 23), to be eliminated from the tetrad nuclei and form micronuclei.

In most of the cells the chromosomes pass to the equator and form regular metaphase plates. In other cells they lie more or less scattered round the whole cell during metaphase II. The last-mentioned cells often seem to develop restitution nuclei and micronuclei. As in the first division, fragments appear in a few cells. These fragments may either

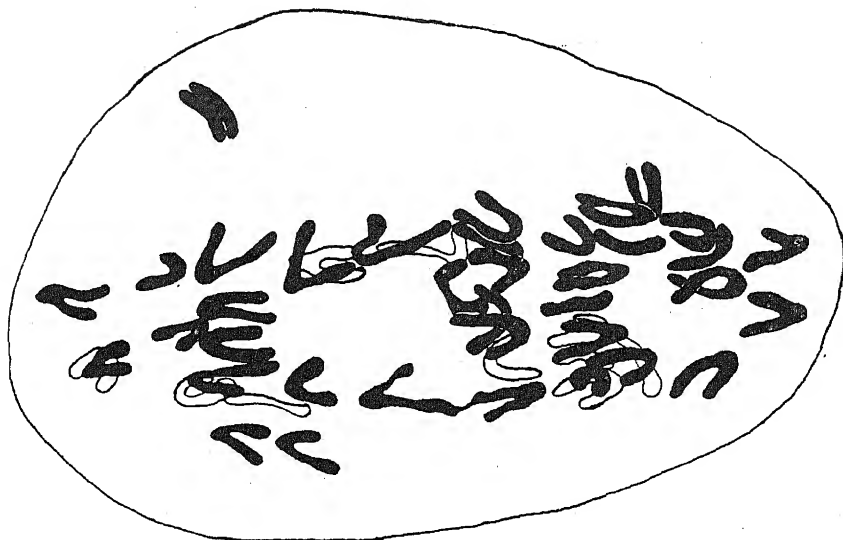


Fig. 24. A cell with unreduced chromosome number during anaphase II. —  
× ca. 1950.

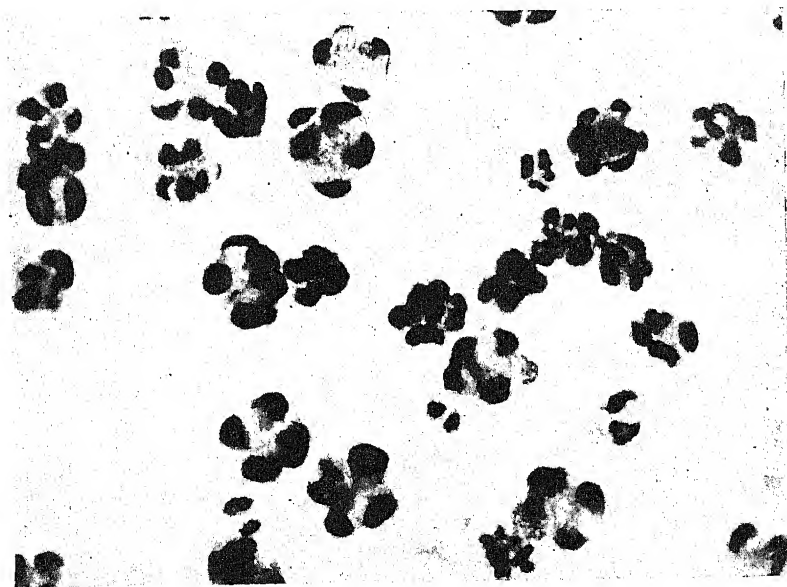
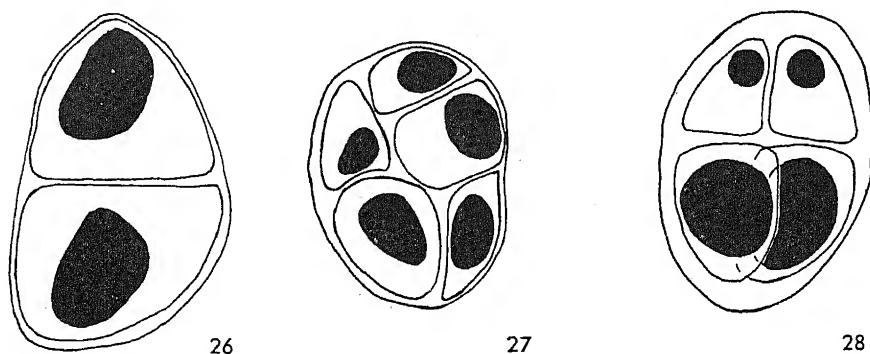


Fig. 25. »Pollen tetrads» from the asyndetic spruce. The variation in size among the pollen tetrads is remarkable and seems to admit of being put in association with the abnormality caused by stickiness. — × 120.

split and be included in the tetrad nuclei or remain unsplit in the equatorial plane.

A characteristic feature of cells with stickiness is, as in the first division, the presence of a number of chromatin clumps and fragments with chromatin bridges. However, a rather large number of free chromatin bridges seem to occur during the second division. Still, there are not so many unchanged or normal chromatids. The amount of chromatin may vary but is largely the same in the two daughter-cells. In solitary cells the chromatin masses in one or both daughter-cells seem to divide rather normally. Most frequently, however, cells characterized



Figs. 26—28. »Tetrads» with variable number of cells and nuclei in the asyndetic spruce. —  $\times$  ca. 650.

by stickiness give rise to one or more micronuclei, which probably degenerate.

Cells in which the heterotypic division has failed to appear divide normally if all the chromatids are double. If some of the chromatids are single, these remain on the equatorial plate during anaphase and form micronuclei. A rare case of two chromatids with an undivided centromere during anaphase II is reproduced in Fig. 24. It is also possible that this univalent is on the way to the equator to divide there.

The »tetrads» in the asyndetic spruce are very irregular (Figs. 25—28). The number of nuclei and cells varies remarkably per »tetrad», as is evident from the illustrations as well as the following figures:

No. of nuclei and cells per »tetrad»:	2	3	4	5	6	7	Total
No. of »tetrads»:	8	24	298	36	6	3	375

A large number of the »tetrads» contain, as expected, extra micronuclei, the three first groups containing the most.

In the largest »tetrad» group the distribution of micronuclei is as follows:

No. of micronuclei per »tetrad»	0	1	2	3	4	5	6	7	8	Total
No. of »tetrads» . . . . .	156	65	51	21	7	3	3	1	1	298

The number of microsporocytes with an unreduced chromosome number has decreased during the second anaphase, which is to be seen from the number of dyads as compared with the number of restitution

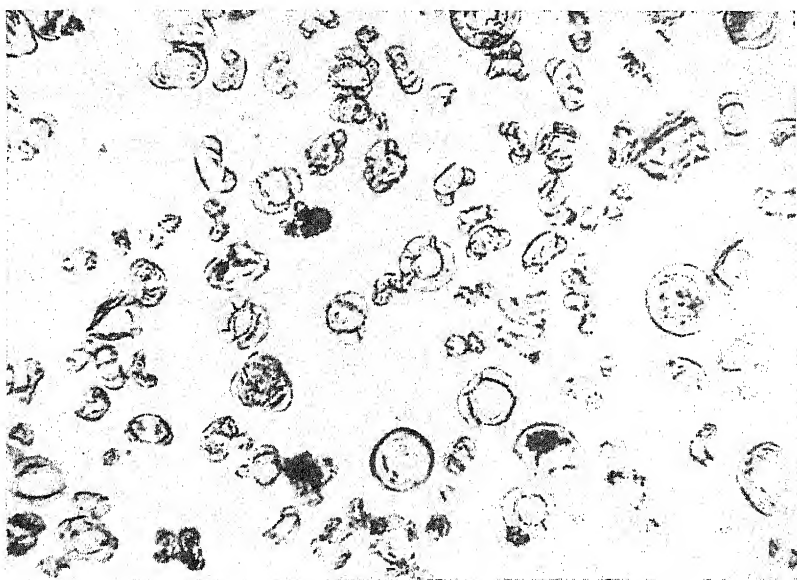


Fig. 29. Pollen from the asyndetic spruce. —  $\times 120$ .

nuclei in the interkinesis stage. This decrease was due to the fact that these pollen mother-cells, unreduced during interkinesis, had contained single chromatids. During the second division such cells showed lagging chromosomes, which in most cases resulted in the arising of »triads» with nuclei of different size or »dyads» with micronuclei. Monads with only one nucleus were not observed. The remaining »triads» probably arose because one of the daughter-cells divided during the second division while the other daughter-cell formed a restitution nucleus. Dyads without micronuclei also occasionally exhibit variable nucleus-size. Two of the eight dyads contained respectively one and three extra micronuclei. The numbers of extra micronuclei in the 24 triads were:

No. of extra nuclei per triad:	0	1	2	3	4	5	Total
No. of triads: . . . . .	8	6	3	4	2	1	24

In the 36 pentads the number of extra micronuclei was as follows:

No. of extra nuclei per pentad:	0	1	2	3	Total
No. of pentads: . . . . .	26	7	2	1	36

Only one of the hexads contains a micronucleus. Stickiness and the irregular chromosome distribution in the asyndetic spruce give rise to a very irregular pollen, which varies both in size and form (Fig. 29).

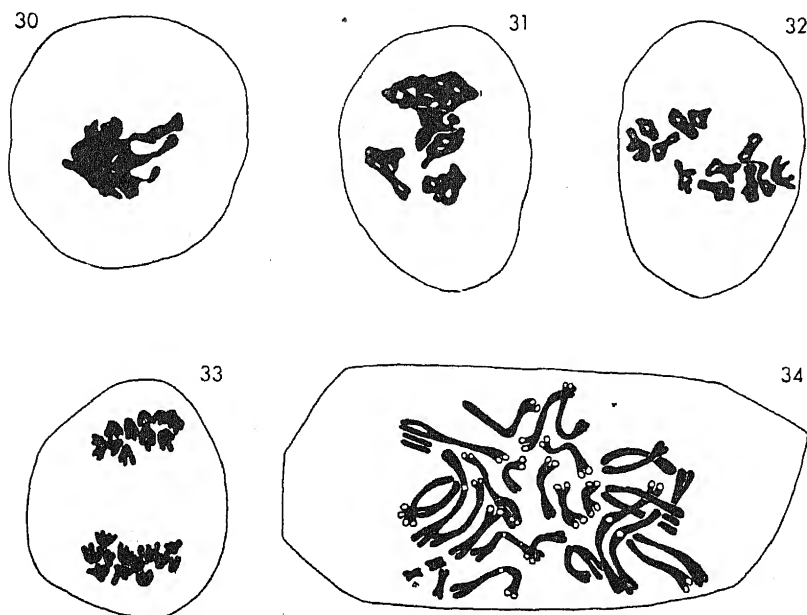
## VI. THREE CASES OF STICKINESS.

The expression »sticky chromosomes» was first employed by BEADLE (1932) to denote a special abnormality of the chromosomes. This malformation consists in the chromosomes forming fused chromatin masses during meiosis and thus losing their individuality (cf. also BEADLE, 1937; JOHNSON, 1944; DARLINGTON and LA COUR, 1940, 1945). Sticky chromosomes, moreover, seem to be considerably smaller than normal chromosomes. BEADLE found in maize a single recessive gene which caused stickiness in somatic divisions as well as to a still higher extent in meiotic divisions. As already mentioned, similar abnormalities occur in solitary microsporocytes in the asyndetic spruce. Stickiness was also found in another three spruces. In pollen mother-cells derived from these trees the chromosomes are fused into more or less irregular chromatin masses. The prophase stages are specially difficult to distinguish, the chromosomes in all these stages being drawn out into chromatin threads or coalesced into chromatin clumps. All kinds of transitional forms between normal or almost normal meiosis and complete stickiness appear.

In Figs. 30 and 31 two cells with pronounced stickiness are represented. Fig. 32 illustrates a cell having a normal or nearly normal meiosis. Each of these pollen mother-cells contains 12 bivalents, although these *are very powerfully contracted*. Of more occasional occurrence are cells with considerable fragmentation. This anomaly is in the asyndetic spruce more characteristic of cells exhibiting stickiness. On the other hand, asymmetric »bivalents» and »multivalents» with abnormal chiasmata occur. An apparently normal anaphase I with *greatly contracted chromosomes* is illustrated in Fig. 33. After anaphase I there usually follows a normal binuclear interkinesis. More

sporadically cells are found with three nuclei, nuclei of very variable size, or restitution nuclei.

The second division, like the first, shows different transitional forms. About half of the tetrad nuclei are of variable size, and micronuclei occur in most of the cells. After the second division several microsporocytes exhibit signs of degeneration.



Figs. 30—31. Cells with sticky chromosomes. — Fig. 32. Almost normal metaphase I in »sticky spruces». — Fig. 33. Anaphase I with greatly contracted chromosomes in a »sticky spruce». — Fig. 34. Somatic chromosomes from root-tips of offspring from the asyndetic spruce. Metaphase in a plant with  $2n = 24 + 2f$  (four chromosomes are furnished with trabants). — Figs. 30—31,  $\times$  ca. 1000. Fig. 34,  $\times$  ca. 1650.

Two of these three spruces are derived from Brunsberg and one from Höljes. The growth locality of the spruces from Brunsberg is situated 70 metres above sea-level, while that of the spruce from Höljes is about 500 metres above sea-level. For fixation purposes the buds were collected direct from the trees. At the time of meiotic division in the spruces, which in 1945 occurred in Värmland between April 12 and 18, great differences in day and night temperatures are not uncommon. Especially on southern slopes the temperature may rise to between  $+15$  and  $+20^{\circ}$  C. on sunny days and fall during the night to between

— 10 and — 15° C. At Brunsberg a minimum temperature of — 8 to — 8,4° C. was registered on a couple of nights during the period in question. On the other hand, the temperature during the day kept between + 9,8 and + 14,1° C.

The possibility cannot therefore be ruled out that these cases of stickiness were caused by cold shock. In support of this hypothesis an experiment may be adduced that was carried out at Brunsberg on April 19, 1945. Branches bearing male inflorescences from No. 112 Brunsberg, which had been forced for twenty-four hours in a greenhouse at a temperature of + 18° C., were placed for the night out in the open. The minimum temperature during the night was registered at — 8,4° C. At the time the branches were moved out the pollen mother-cells were in process of division, and a control examination showed the majority to be in metaphase I. When the branches were taken in the following morning, most of the cells, after fixation, showed stickiness. The disturbances of meiosis following the cold treatment were at least as powerful as in the three »sticky spruces». Controls left in the greenhouses all showed normal meiosis.

Militating against the assumption that the stickiness in the three spruces mentioned is a consequence of temperature changes is the fact that two spruces from the same locality at Höljes and three from Brunsberg, which were fixed at the same time as the spruces found with stickiness from these localities, did not present any meiotic disturbances whatever.

From experience we know that the seed-setting of forest trees is especially poor in high positions, which means that self-juvenescence is jeopardized to a high degree within such forest areas. Now, does this poor seed-setting depend to some extent on modificative disturbances in the course of meiosis or to unfavourable climatical conditions during the pre- and post-fertilization periods? Unfortunately, we are not able at present to answer this and similar questions.

## VII. PROGENY OF No. 181 AFTER FREE FLOWERING.

The 62 surviving plants after wind pollination of No. 181 exhibit highly varying height growth, growth habit, needle-length and stomasize (cf. Tables 3 and 4). The variation within this progeny-group is strikingly large compared with that within the other progeny-groups of the same age from normal spruces. In habit some of the plants are reminiscent of witch-knot. This habitual variation is in all probability

TABLE 3. *Comparison between stomatal size, plant height and some chromosome numbers in progeny of spruce 181 after free flowering (1 scale division =  $2.47 \mu$ ).*

Plant No.	Stomatal length	Stomatal breadth	Plant height in mm.	$2n$
1	$20.97 \pm 0.11$	$13.68 \pm 0.08$	70	
2	$20.55 \pm 0.13$	$12.63 \pm 0.09$	102	
3	$20.91 \pm 0.13$	$12.97 \pm 0.07$	81	
4	$21.28 \pm 0.10$	$12.69 \pm 0.09$	110	
5	$21.68 \pm 0.13$	$12.35 \pm 0.09$	93	
6	$21.38 \pm 0.17$	$12.79 \pm 0.11$	98	
7	$21.36 \pm 0.12$	$12.84 \pm 0.08$	95	
8	$20.83 \pm 0.13$	$13.50 \pm 0.09$	123	
9	$20.80 \pm 0.11$	$13.52 \pm 0.09$	122	
10	$21.48 \pm 0.13$	$15.21 \pm 0.11$	50	
11	$20.44 \pm 0.09$	$13.58 \pm 0.10$	114	
12	$21.24 \pm 0.12$	$13.18 \pm 0.09$	140	$24 \pm$
13	$21.55 \pm 0.15$	$14.75 \pm 0.18$	78	
14	$20.45 \pm 0.12$	$13.24 \pm 0.09$	70	
15	$20.20 \pm 0.13$	$13.29 \pm 0.10$	65	
16	$19.30 \pm 0.10$	$13.63 \pm 0.12$	88	
17	$20.49 \pm 0.11$	$13.11 \pm 0.11$	128	24
18	$20.32 \pm 0.11$	$13.08 \pm 0.09$	130	
20	$20.89 \pm 0.13$	$13.17 \pm 0.11$	65	24
21	$20.19 \pm 0.08$	$13.10 \pm 0.08$	104	
22	$20.45 \pm 0.12$	$13.12 \pm 0.10$	87	
23	$23.27 \pm 0.11$	$15.56 \pm 0.17$	110	
24	$20.91 \pm 0.13$	$13.58 \pm 0.09$	121	
25	$20.12 \pm 0.10$	$13.24 \pm 0.10$	60	
26	$19.91 \pm 0.11$	$13.08 \pm 0.11$	35	
27	$22.30 \pm 0.16$	$15.06 \pm 0.11$	66	
28	$20.55 \pm 0.16$	$13.18 \pm 0.09$	90	
29	$19.62 \pm 0.08$	$13.84 \pm 0.11$	48	
30	$22.90 \pm 0.13$	$15.10 \pm 0.10$	122	
31	$19.40 \pm 0.10$	$13.17 \pm 0.12$	95	
34	$21.78 \pm 0.12$	$13.29 \pm 0.06$	130	24
35	$20.55 \pm 0.12$	$13.58 \pm 0.11$	30	
36	$19.97 \pm 0.11$	$13.35 \pm 0.11$	122	
37	$20.29 \pm 0.12$	$13.00 \pm 0.12$	98	
38	$20.77 \pm 0.15$	$13.09 \pm 0.11$	47	
39	$21.31 \pm 0.14$	$13.85 \pm 0.12$	103	24
40	$21.18 \pm 0.12$	$13.70 \pm 0.10$	137	24
41	$19.58 \pm 0.10$	$13.49 \pm 0.11$	63	
43	$20.90 \pm 0.16$	$13.22 \pm 0.13$	64	
46	$20.94 \pm 0.18$	$13.67 \pm 0.12$	112	24
47	$20.88 \pm 0.13$	$13.88 \pm 0.11$	125	24
48	$21.16 \pm 0.11$	$12.68 \pm 0.10$	126	24

	Stomatal length	Stomatal breadth	Plant height in mm.	2n
Plant No. 49 .....	21,73 $\pm$ 0,17	13,75 $\pm$ 0,13	110	24
50 .....	20,41 $\pm$ 0,12	14,12 $\pm$ 0,09	118	
51 .....	20,05 $\pm$ 0,11	13,57 $\pm$ 0,11	102	
52 .....	21,09 $\pm$ 0,12	13,85 $\pm$ 0,11	102	
53 .....	20,80 $\pm$ 0,12	14,27 $\pm$ 0,09	110	24 $\pm$
54 .....	20,25 $\pm$ 0,10	13,99 $\pm$ 0,10	142	24 $\pm$
55 .....	21,02 $\pm$ 0,12	13,28 $\pm$ 0,10	85	
56 .....	20,28 $\pm$ 0,12	13,30 $\pm$ 0,10	120	24 $\pm$
58 .....	20,90 $\pm$ 0,13	13,60 $\pm$ 0,10	87	
59 .....	20,89 $\pm$ 0,12	13,34 $\pm$ 0,09	96	24 + 2ff.
60 .....	20,69 $\pm$ 0,17	14,46 $\pm$ 0,15	76	
62 .....	22,33 $\pm$ 0,12	15,75 $\pm$ 0,15	80	
63 .....	20,26 $\pm$ 0,11	13,20 $\pm$ 0,10	140	24
64 .....	21,54 $\pm$ 0,11	15,06 $\pm$ 0,21	110	
65 .....	21,15 $\pm$ 0,13	13,25 $\pm$ 0,10	75	
66 .....	20,88 $\pm$ 0,12	13,65 $\pm$ 0,09	93	
67 .....	20,88 $\pm$ 0,11	13,58 $\pm$ 0,10	111	
68 .....	20,69 $\pm$ 0,11	13,72 $\pm$ 0,09	118	
69 .....	20,70 $\pm$ 0,09	12,87 $\pm$ 0,11	50	
70 .....	20,00 $\pm$ 0,08	13,49 $\pm$ 0,09	80	

caused by the genotypic constitution. The majority of the plants, however, have a normal growth habit and show ordinary growth in height. All the progeny-plants were root-fixed during the summer of 1945 on five different occasions. On account of the nature of the chromosomes it has unfortunately only been possible to count a few plants.

In *Picea Abies* the chromosomes are long and often lie entangled in the somatic cells during metaphase, with the result that it is usually impossible to determine the somatic number. Similar difficulties have been experienced by K. and H. J. SAX (1933) in attempts to determine the chromosome number in root-tips of Conifers.

Among the progeny of No. 181 it has been possible to count the chromosomes of 15 plants. Four of these counts are uncertain, however. Annoyingly enough, the chromosome number of some of the habitually deviating plants have not admitted of being counted, as will be seen from Table 3. In one of the plants with an ordinary growth-habit and normal stoma-size one plate has 24 chromosomes + two fragments (Fig. 34). Other plants counted have 24 chromosomes.

At the examination of the stomata in the progeny 35 cells were measured on each of three needles per plant. The needles were taken from the top of the plants. With the exception of two plants, in which 100 stomata were measured, 105 observations were accordingly made

per plant. Thus, altogether 6.500 measurements were made. On an average taken over the whole progeny the length of the stomata is  $27,78 \pm 0,0023$  scale divisions and the corresponding cell-breadth  $13,57 \pm 0,0016$  divisions (1 division =  $2,47 \mu$ ). The variation in cell-size of the individual plants is given in Table 3. Four plants deviate very much in both breadth and length of cell and three plants in cell-breadth. In these plants the length and breadth of the cells are on an average 9,2 and 12,1 % larger than the corresponding mean of all the progeny of No. 181.

TABLE 4. *Analysis of variance. Length of stomata in progeny obtained from spruce 181 after free flowering.*

Variation	Df.	Sum of squares	Mean square	Quot.
Between plants .....	61	12.494,60	204,83	124,29***
Within plants (= error) .....	6438	10.610,11	1,648	P < 0,001
Total	6499	23.104,80		
Between needles within plants .....	124	1.363,66	11,0	6,67***
Within needles .....	6314	9.246,45	1,470	P < 0,001
Total (within plants)	6438	10.610,11		
<i>Stomatal breadth in same progeny.</i>				
Between plants .....	61	3.360,27	55,09	45,23***
Within plants (= error) .....	6438	7.840,53	1,218	P < 0,001
Total	6499	11.200,80		
Between needles within plants .....	124	1.392,75	11,23	9,22***
Within needles .....	6314	6.447,78	1,021	P < 0,001
Total (within plants)	6438	7.840,53		

In Table 4 the variation in stoma-size has been divided into *variation between plants* and *between needles within plants*. The table shows that there is a significant difference in cell-size between plants as well as between needles within plants. It is surprising that the last-mentioned variation is significant, since, as already pointed out, the needles were taken from the top of the plants.

## VIII. CONCLUSIONS AND DISCUSSION.

With the exception of *Sequoia sempervirens*, *Pseudolarix amabilis* and *Juniperus chinensis Pfitzeriana* no stabilized polyploid species have been found among Pinaceae and Cupressaceae. These three species all have the tetraploid chromosome number and thus constitute the only cases within these plant-groups, in which polyploids and environment have formed a favourable combination. Probably polyploid individuals occur now and then in other allied genera, although they have been unable to survive as stabilized species. It is of some interest to note that the three polyploid species found among coniferous trees seem to be autopolyploids (cf. K. and H. J. SAX, 1933). In nature no allopolyploid species or individuals of spontaneous origin are at present known among the Conifers, although SYRACH LARSEN and WESTERGAARD (1938), by crossing *Larix decidua* ( $2n = 24$ ) to *L. occidentalis* ( $2n = 24$ ), succeeded in obtaining a triploid hybrid ( $2n = 36$ ) which, since two different species are concerned here, must be considered to be an allotriploid.

The autotetraploids found among the Conifers would seem to have appeared in two steps, probably through an unreduced ovum in a diploid tree being fertilized by a haploid pollen-grain from another diploid tree and in course of time giving rise to a triploid plant. This triploid in its turn probably produced an unreduced egg-cell that was fertilized by a haploid male gamete. If the tetraploids had arisen in one step, this would imply that an unreduced macrospore had been fertilized by an unreduced microspore which had likewise arisen. However, the chance of two unreduced gametes arising and fertilizing each other must be said to be infinitely small. Moreover, a fertilization of that kind would probably cause a disturbed development of the seeds, as during seed development three different tissues are in very intimate connection with each other, viz. the investing somatic tissues of the mother-tree and the embryo and endosperm tissues (cf. MÜNTZING, 1933, 1936).

After normal fusion of haploid gametes in diploid Conifers the relation between the chromosome numbers of these tissues is expressed by the ratio 2 : 2 : 1, in the order given. If it were possible for two unreduced gametes in diploid coniferous trees to fuse with each other, the corresponding ratio would be 2 : 4 : 2, which may cause physiological as well as morphological disturbances in the connection between embryo- and endosperm-tissues and the surrounding diploid tissues. It is of course also conceivable that triploid plants may arise as a result of a haploid egg-cell being fertilized by an unreduced pollen-grain. That

seems in fact to have been the case in the triploid larch hybrid obtained (cf. SYRACH LARSEN and WESTERGAARD, 1938). Among the Angiosperms, however, in crosses between diploid individuals, triploid progeny seem more likely to arise when unreduced egg-cells are fertilized by haploid pollen-grains than in the case in the other direction. These differences in seed development depend, according to MÜNTZING, in all probability on numerical changes in the chromosome sets as between embryo, endosperm and the surrounding somatic tissues of the maternal plant (cf. MÜNTZING, 1933, 1936).

The relation in chromosome numbers between the endosperm and the investing somatic tissues of the mother-plant frequently seems to be of importance for the development of the endosperm. Probably, however, different species react differently to disturbances between these tissues. The first-mentioned conclusion corresponds well with the results reported by COOPER and BRINK (1940) respecting the development of the endosperm and the innermost cell-layer of the integument after self-fertilization in *Nicotiana rustica* and after the cross *N. rustica* ( $n=24$ )  $\times$  *N. glutinosa* ( $n=12$ ). In that case the endosperm and investing tissue develop quite differently after self-fertilization and crossing. After selfing the endosperm and its surrounding cell-layers grow normally (about 77 % of the volume of the ovule consists of endosperm and 23 % of »nucellus»), while the relation between these tissues in the hybrid *N. rustica*  $\times$  *N. glutinosa* is essentially changed (only about 25 % is endosperm while 75 % of the volume of the ovule consists of »nucellus»). In the last-mentioned case the innermost cell-layer of the inner integument produces a considerable number of cells, which in course of time completely enclose the endosperm. This results in a rupture of the funicle, and communication between endosperm and placenta ceases. This type of abortion may be associated with the change in the chromosome relations between endosperm and surrounding somatic tissue. At inbreeding the chromosome ratio as between embryo, endosperm and surrounding somatic tissue is the normal, 2 : 3 : 2, or in this case 4 : 6 : 4, while the ratio as between the same tissues after the cross mentioned is 3 : 5 : 4.

Some abnormal conditions in the development of endosperm and antipodal cells after crossing *Hordeum jubatum* ( $n=14$ ) to *Secale cereale* ( $n=7$ ) have been described by BRINK and COOPER (1944) and COOPER and BRINK (1944). These disturbances, however, may also depend on other causes than solely changes in the chromosome sets between neighbouring tissues.

Besides the cases already mentioned, polyploid forms may also occur as a result of asyndesis. This implies nothing fundamentally new in comparison with the cases just described, as in that case, too, the polyploids must be considered to arise through the union of an unreduced and a haploid gamete or of two unreduced gametes. However, the possibility of unreduced gametes appearing is considerably greater in asyndetic trees than in trees having a completely normal reduction division. It cannot therefore be ruled out that a number of triploid plants may appear in the vicinity of an asyndetic tree. These triploids will then have many opportunities of crossing either with neighbouring diploids or with triploids and aneuploid half-sibling plants in the vicinity. Such crosses ought to result in the production of, for instance, tetraploids and new triploids.

Although the Conifers undoubtedly suffer from self-sterility, it has been shown that a certain amount of seed-setting may be expected after self-fertilization of, e. g., spruce and pine (cf. DENGLER, 1932, 1939). The polyploids that arise, like other polyploids among the Angiosperms, should be more self-fertile and less susceptible to inbreeding depression than the corresponding diploid Conifers. In the spruce, however, some degree of metandry (protogony) seems to exist (SYLVÉN, 1910; SYRACH LARSEN, 1937). This difference in flowering-times of female and male flowers on the same tree may therefore completely or partially prevent self-fertilization. Consequently, cross-fertilization may be considered the normal and probably the only possible means of fecundation at wind-pollination of the spruce. If the incomplete or completely absent chromosome pairing during meiosis is conditioned by genes, single asyndetic individuals ought to be able to »segregate out» continuously in the populations.

Certainly it is probable that a large number of the progeny of an asyndetic tree are marked by disturbed meiosis and low pollen fertility and seed production, whereby the spread of the new polyploids is rendered more difficult. None the less, there have been possibilities for stabilized polyploid species to originate among our native Conifers as well, this not least of all owing to the fact that the Conifers belong to a demonstrably very old group of plants. There is accordingly reason to think that polyploids might also have arisen by a vegetative method. Tetraploid cells and sectors have been found in the ordinary diploid tissue of several plants, and such cells may also occur in the Conifers. Should these tetraploid sectors give rise to a branch with a collection of tetraploid inflorescences, a number of diploid gametes must thus be the result.

It is evident that changes in the chromosome numbers have been of little account in the differentiation of most coniferous genera. No stabilized polyploid species have so far been found among our most common Conifers, e. g., spruce, pine and larch. Thus, it is a plausible presumption that existing diploid Conifers are best adapted to present-day environmental conditions, and that as a rule their chromosome number may be considered to be that constituting the maximum of the species in question.

In spite of the absence of stabilized polyploid species among, for instance, *Picea* and *Pinus* solitary finds of triploid and tetraploid individuals would have been expected. No trees with morphological characters deviating from the normal type and suggestive of polyploidy have been found in middle-aged and older stands of Conifers in spite of numerous examinations having been made of, among others, the seed-setting in different coniferous trees. Hence the frequency of any possibly existing polyploid spruces with normal or more than normal growth capacity must be very low, or else polyploid zygotes within certain genera must have already died during the development of the embryo owing to disturbances in the chromosome-number relation between embryo, endosperm and surrounding somatic tissue. The polyploids may also have consisted of dwarfs or stunted trees, which have died or are cleared away at thinnings of the stands. That in all probability the frequency of polyploid Conifers is remarkably low may partly be due to the fact that the method of fertilization does not allow of polyploids or corresponding diploids being sexually isolated, or partly to the above-mentioned chromosomal disturbances between different embryonal tissues of the polyploid zygote. Changes in cell-size or in rate of cell-division may be quite sufficient to prevent normal seed development or to produce so-called somatoplastic sterility (cf. COOPER and BRINK, 1940, 1944).

As regards the dissemination of chromosome-altered forms, this must depend to a large extent on the mode of fertilization. Polyploid cross-fertilizers that are not isolated from original diploid types in the vicinity will soon, according to MÜNTZING (1933), be broken down through chromosome elimination during a series of back-crossings with the original diploid material. As different species react in different ways to chromosome doubling, it is not excluded that, for instance, polyploids of *Picea Abies* also have a vitality inferior to that of the corresponding diploids.

According to SAX's theory (cf. SAX, 1932), the absence among Coni-

fers of polyploids with a high number of interstitial chiasmata per bivalent may to some extent depend upon an irregular distribution of homologous chromosomes during meiosis in any autopolyploids that may arise. In *Picea Abies* the average number of chiasmata per bivalent is 2.7. Moreover, most of these chiasmata are interstitial. Hence it is not unlikely that chromosome pairing at meiosis in tetraploid spruces will result in a very intimate and general formation of quadrivalents. As a rule a quadrivalent formation of this kind would cause an irregular distribution of homologous chromosomes to the two poles, which might result in extreme gamete sterility.

The pollen fertility in *Pseudolarix* and the tetraploid *Juniperus chinensis Pfitzeriana*, however, affords no support for the hypothesis that quadrivalent configurations during meiosis would cause non-disjunction (cf. K. and H. J. SAX, 1933). The pollen sterility in *Pseudolarix* is less than 5 % and in the tetraploid *Juniperus* 6 %, which is lower than in the corresponding diploids. The number of chiasmata per bivalent is 2.1 in *Pseudolarix* and 2.2 in *Juniperus communis* ( $2n = 22$ ).

The meiotic division in *Picea Abies* seems to be normal as a rule, though irregularities occur. Three »sticky trees» have been encountered. These observed cases of stickiness, however, may be of modificative nature and in that case caused by the great changes of temperatures between day and night. In addition to these anomalies one asyndetic spruce has been discovered. *This case of asyndesis seems to be the only one that has hitherto been observed among the Gymnosperms.*

Several cases of asynapsis are known within the Angiosperms and some in the animal kingdom, e.g., among *Drosophila pseudo-obscura*, *Pygaera* hybrids (cf. DARLINGTON, 1937, p. 414) and *Triton* (BÖÖK, 1945). A summary of previously examined instances of asynapsis has been presented by PRAKKEN (1943), which has been supplemented by JOHNSON (1944). PRAKKEN distinguishes the following groups of synapsis: (1) Asynapsis due to the action of a distinct gene or genes (or some slight structural change). (2) Asynapsis caused by loss of a chromosome pair. (3) Asynapsis induced by external conditions. (4) Asynapsis as a normal process in apomictic organisms. (5) Asynapsis depending upon mechanical chromosome conditions (structure, number). (6) Asynapsis in species hybrids.

The instance of asyndesis described here in *Picea Abies* may in all probability be assigned to group 1. More or less certain cases of gene-conditioned asyndesis are known. Genetically governed asyndesis has been described in, among others, *Zea* (BEADLE and MCCLINTOCK, 1928; BEADLE, 1930, 1933), *Nicotiana tabacum* (CLAUSEN, 1931), *Hordeum* (EKSTRAND, 1932), *Datura* (BERGNER, CARTLEDGE and BLAKESLEE, 1934), *Nicotiana sylvestris* (GOODSPEED and AVERY, 1939), *Alopecurus myosuroides* (JOHNSON, 1941, 1944), *Secale* (PRAKKEN, 1943), *Rumex* (LÖVE, 1943), and *Triticum vulgare* (LI, PAO and LI, 1945).

BEADLE has shown earlier that there are recessive genes which

cause asynapsis. In addition genes occur that induce supernumerary divisions in the gametophytes, and such as to some extent suppress cell-wall formation. CLARK (1940) even found a gene, in maize, that diverged the heterotypic spindle instead of converging it. The result of this will be that the chromosomes do not pass to the poles to form normal telophase nuclei but remain scattered in the cells and give rise to many micro-nuclei. This mono-factorial recessive asynapsis shows widely differing characteristics in different objects. The relative number of univalents, rod and ring bivalents, and chiasmata varies from case to case. Variation is likewise exhibited by the chromosome pairing during early prophase, the behaviour of the univalents during the heterotypical division, and the number of restitution nuclei and functional dyad pollen grains, etc. within different materials.

Complete pairing of chromosomes during pachytene (desynapsis) has been observed in individuals of *Zea mays* (BEADLE, 1930, 1933), *Crepis capillaris* (HOLLINGSHEAD, 1930; RICHARDSON, 1935), *Nicotiana sylvestris* (GOODSPEED and AVERY, 1939), triploid *Allium amplexans* (LEVAN, 1938, 1940), *Secale cereale* (PRAKKEN, 1943), *Triticum vulgare* (LI, PAO and LI, 1945), and *Picea Abies* (in the present work). On the other hand, no zygotene and pachytene pairing seems to take place in asynaptic *Rumex acetosa* (YAMAMOTO, 1934) and there is incomplete prophase pairing in, e. g., asynaptic dwarf fatoid oats with 40 chromosomes (HUSKINS and HEARNE, 1933) and probably in *Alopecurus myosuroides* (JOHNSON, 1941).

In all asyndetic individuals showing more or less complete zygotene and pachytene pairing the separation of the paired chromosomes generally takes place gradually. The time at which this separation takes place, however, appears to vary in different asyndetic materials and even between individuals of the same species. In the above-described *Picea* instance the paired chromosomes already begin to fall apart at the end of pachytene. During the middle and late diplotene stage only a few bivalents occur, or about the same bivalent frequency as was stated for metaphase I.

The same is to some extent the case in maize (BEADLE, 1930, 1933) and wheat (LI, PAO and LI, 1945). According to the last-named Chinese scientists, the apparently paired chromosome threads in some desynaptic wheat plants separate at pachytene, while in other wheat plants the corresponding chromosome separation does not take place until the diplotene phase. In *Allium amplexans* (LEVAN, 1940) and *Secale cereale* (PRAKKEN, 1943) the paired members are kept together during diplotene by relational coiling, and even during diakinesis many pairs of univalents appear.

Several hypothesis have been advanced to explain the separation of the homologous chromosomes of asynaptic plants during prophase. According to DARLINGTON, the failure of chiasma formation may be due to the fact that at leptotene the chromosome separation is relatively far advanced, or that the beginning of prophase is relatively delayed. In both cases, accordingly, it is presumed that the chromosome threads have already parted during leptotene, which is considered to prevent a normal attraction of homologous chromosome threads. In partial asynapsis the peculiar situation must arise, according to DARLINGTON, that only some of the chromosomes divide lengthwise during leptotene, while other chromosome threads remain undivided.

HUSKINS and SMITH (cf. HUSKINS, 1932, p. 6), however, seem to have observed in *Fritillaria meleagris* that certain rather considerable portions of the chromosomes are longitudinally divided during the early heterotypic prophase, and that a complete pairing takes place between undivided homologous chromosome segments, while the corresponding split sections do not pair, which in itself ought to cause partial asynapsis. From that point, however, it is not a far step for the longitudinal division to take in the whole or the greater part of certain chromosomes, as at the time of pairing homologous chromosomes have probably not yet managed to find each other.

Another theory has been presented by SAX and SAX (1935). According to these scientists, one of the prerequisites for chromosome pairing is that the chromosome threads are despiralized before the zygotene phase begins, since a high degree of spiralization seems to prevent pairing. During a long prophase the chromosome threads therefore have greater possibilities of despiralizing before the beginning of the zygotene phase than during a short and very rapidly passing early prophase.

Thus, these two hypotheses, presupposing as they do that the chromosomes have been prevented from pairing during prophase, do not hold in all observed cases of desynapsis.

In the asyndetic spruce the behaviour of the univalents during metaphase I is of some interest, as it also is in the majority of asyndetic Angiosperms. When bivalents are altogether lacking in the pollen mother-cells, which is the case in one-third of the cells of the asyndetic spruce, the univalents preferably pass straight to the poles. This is inferable from the behaviour of the bivalents in other neighbouring cells during early metaphase I. *Thus, in cells with solely univalents the latter are distributed to the poles during metaphase I.* On the other hand, if there are one or more bivalents in a pollen mother-cell, a

greater or a smaller number of univalents are often drawn to the equator and are forced to range themselves in an equatorial plate in common with the bivalents.

The different behaviour of the univalents in these two cases seems to be explained by ÖSTERGREN's centromere theory (unpublished; cf. ÖSTERGREN and PRAKKEN, 1946). ÖSTERGREN presumes that at a somatic mitosis the chromosomes during metaphase have a centromere consisting of the sum of two anaphase-centromeres. This undivided centromere is equally attracted by both poles and therefore goes to the equator. When the centromere has divided, each of its halves is directed toward one pole only and is accordingly attracted solely by this pole. According to ÖSTERGREN, during metaphase I the univalents of, for instance, the asyndetic spruce will be in a state *between singleness and doubleness*. At the earliest period of metaphase I, when the centromeres of the univalents are incompletely polarized, the univalents migrate to one of the poles. On the other hand, when the centromeres of the univalents have become doubled or polarized the univalents pass to the equator.

Only a small number of bivalents occur in the asyndetic spruce. Judging from the length of the chromosomes and the positions of the centromeres, pairing between homologous chromosomes will as a rule have taken place among the bivalents. Still, we ought not to leave out of account the possibility of non-homologous pairing. LEVAN (1942, 1945) has found that the chromosome threads are distinctly paired during prophase in haploid rye and haploid sugar-beet. Non-homologous pairing has already been observed by MCCLINTOCK (1933) in maize. In addition LEVAN found several symmetric bivalents in his haploid material. The most interesting find in LEVAN's studies of rye and sugar-beet, however, is that almost all chiasmata are distributed at random or show good agreement with the POISSON's distribution, which shows that it cannot be a question of *solely chiasmata formed between duplicated chromosome segments*. From these results it seems bold — except in cases of complete pachytene pairing (or desynapsis) — to assume unreservedly that all observed bivalents of asyndetic plants are the products of homologous pairing.

As was earlier pointed out, a number of irregularities occur during the first division in the asyndetic spruce, such as irregular distribution of univalents, non-disjunction, premature centromere division or divided univalents, fragmentation, stickiness, and misdivision. Difficulties generally arise at the separation of chiasmata during anaphase I, and in

consequence a relatively high percentage of chromatin bridges occur in relation to the number of bivalents. These anomalies during the heterotypic division result in an almost equally irregular homotypic division, with chromosome elimination and greatly varying chromosome numbers in the gametes. As expected, this ultimately results in an abnormally high pollen sterility. Only two per cent morphologically good pollen have been produced. Still, this low percentage probably includes a few unreduced gametes that are capable of functioning. Judging from the seed-setting in the asyndetic spruce, we may venture to conclude that the embryo-sac mother-cells exhibit about the same degree of asyndesis as the pollen mother-cells.

Besides other methods at present being employed to produce polyploids in experimental breeding work on spruce, reciprocal crosses between this asyndetic spruce and normal spruces ought to be carried out to the greatest possible extent, it being conceivable that a polyploid may be obtained here and there as a result of such crossing experiments.

Finally, there may be reason to point out that no greater fundamental differences seem to exist between the above-reported case of asyndesis in a representative of the Gymnosperms and formerly known instances of the same cytological phenomenon within the Angiosperms.

### SUMMARY.

(1) A case of asyndesis in *Picea Abies* has been observed. Comparisons have been made between meiotic divisions in normal spruces and the asyndetic specimen.

(2) Meiotic division seems to be very regular in spruces in general, though exceptions occur. During metaphase I the average number of chiasmata per bivalent is 2.7. No significant difference in chiasma frequency has been observed as between normal spruces. Most chiasmata have an interstitial position and do not terminalize at all or very inconsiderably.

(3) Microsporogenesis proceeds according to the simultaneous scheme in normal spruces and as a rule in the asyndetic spruce.

(4) The zygotene and pachytene pairing in the asyndetic spruce is almost complete.

(5) During metaphase I bivalents occur in the asyndetic spruce in 41 % of the number of cells, and the number of bivalents per cell varies between 1 and 8. In number the bivalents in these cells amount to 19.35 % of the number possible. In all examined pollen mother-cells

the frequency of bivalents is only 11,77 % of the number of possible bivalents. Of this frequency, 10,96 % consist of rod bivalents and 0,81 % of ring bivalents.

(6) Premature centromere division occurs during anaphase I in 4,1 % of the number of pollen mother-cells.

(7) Chromosome distribution to the poles is not altogether at random. The extreme groups 0 : 24, 1 : 23, 2 : 22 and so on are over-represented owing to the fact that the chromosomes show a tendency to appear in groups, which often behave as a unity.

(8) Stickiness has been observed in a number of pollen mother-cells in the asyndetic spruce.

(9) During interkinesis the cells exhibit a varying number of nuclei. In one case a cell with 16 nuclei was observed. Restitution nuclei have been formed in 8,76 % of the number of cells.

(10) The second division as well as the first division are very irregular owing to elimination of chromatids, stickiness and irregular chromosome distribution.

(11) The »tetrads» often contain micro-nuclei, and the morphological pollen fertility is very poor.

(12) Besides being observed in the asyndetic spruce, stickiness has been found in pollen mother-cells of three other spruces.

(13) Progeny obtained from the asyndetic spruce after free flowering show varying height growth, growth habit and stomatal size.

(14) The sparse occurrence of stabilized polyploid species within the Conifers has been discussed as well as various reasons for the fact that no single polyploid tree has been found within *Picea Abies*.

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# "GENE DIVISIBILITY", AS STUDIED BY DIFFERENCES IN BAR FACET NUMBERS IN *DROSOPHILA MELANOGASTER*

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## I. THE PROBLEM.

THE present article is a continuation of studies on the effect of the gene »Exaggeration of Bar» (symbol *Eb*) on which an earlier report has been given (BONNIER, NORDENSKIÖLD and BÄGMAN, 1943). The principal facts concerning this gene which were brought together in this report were the following. *Eb* is a dominant sex-linked lethal which reduces crossing-over between Bar und fused from 2,5 to about 1,5 units. Linkage tests show that *Eb* is situated close to the right of Bar, at a map distance of probably less than 0,1 units. In co-operation with Bar it intensifies the Bar effect. A *B/Eb* female has eyes of the homozygous Bar size, and a *B Eb/+* female has still narrower eyes. *Eb* does thus also show the Bar position effect. When no Bar gene is present an *Eb/+* female has round eyes, but together with Bar it acts as though it were a (lethal) Bar gene. The exact origin of *Eb* is not known with certainty. But the data show that it must have occurred in a female with attached *X*'s homozygous with respect to Bar. As females *B/B* and *B/Eb* are similar in appearance (at least as long as no facet counts are made), it is quite natural that the true moment of occurrence of *Eb* escaped attention. It was in connection with detachments that it was first observed that something had gone wrong. Such detachments did show that they no longer contained *B* but the new gene *Eb* instead, and it was first believed that *Eb* was an allele of *B*. Later, however, cross-overs between *B* and *Eb* were found, but the crossing-over percentage was of the same order of magnitude as the percentage of unequal crossing-over within the Bar region. The salivary chromosomes of females *Eb/+* were found to contain only one of the two segments of Bar. These chromosomes indicated furthermore a slight possibility that an *Eb* chromosome is deficient of one of the faint bands of the Bar segment.

It may thus be concluded that *Eb* occurred as a (spontaneous) mutation from Bar, and that the Bar chromosome from which it originated lost one of its segments probably in connection with the mutation. But the most interesting fact was revealed when the facet counts were made. As it is known that facet number modifiers are very common,

TABLE 1. *Excerpt from Table 11 in BONNIER, NORDENSKIÖLD and BÅGMAN (1943). The number of the strains (leftmost column) does not refer to Table 11, but are given as references to the present paper only. The genotypes of the female show only the genes B and Eb and not other genes (see text).*

Strain No.	Genotype of females	Number of eyes counted	Mean facet number
1	<i>B/Eb</i>	107	84,3
2	<i>BEb<sub>1</sub>+</i>	190	40,6
3	<i>B/Eb</i>	102	90,2
4	<i>B/Eb</i>	116	89,8

the counts were preceded by standardizing isogeneous lines by a procedure of inbreeding (v. i.). When such lines had been established, search was again made for cross-overs between *B* and *Eb* (using *f* and *fu* as marker genes). Thus there were, among others, four different strains in which the females were constituted as follows (disregarding all genes other than *B* and *Eb*): (1) *B/Eb*; (2) *B Eb<sub>1</sub>+*, which occurred through crossing-over in strain 1; (3) and (4) *B/Eb*, which occurred through crossing-over in strain 2 on two different occasions. The facet counts in these four strains gave the results shown in Table 1. (For further details cf. Table 11 in BONNIER, NORDENSKIÖLD and BÅGMAN, 1943.) From an analysis of variance with regard to the comparison between the three strains of *B/Eb* females it was found that whereas there was no statistical difference between the facet numbers of the two recovered strains 3 and 4, 90,2 and 89,8 respectively, the difference between these two on the one hand, and the original strain No. 1 with its 84,3 facets on the other, was statistically very significant. The results did thus show that two lines of *B/Eb* females of which the one had originated from the other through two instances of crossing-over between *B* and *Eb*, but which otherwise must have been isogeneous (or at least were intended to be isogeneous), had different numbers of facets. It seemed then necessary to try to elucidate somewhat further

this very peculiar situation. The present article contains a description of the new experiments.

## II. THE PROCEDURE OF STANDARDIZING ISOGENEOUS STRAINS.

The principles of the procedure by which standardized isogeneous strains were synthesized has been put together in the preceding article (BONNIER, NORDENSKIÖLD and BÅGMAN, 1943). As it is, however, of importance to be in a position to judge — if possible — how far the isogeneity really reaches, all known details concerning the relevant strains will be given here. Unfortunately the exact number of inbreeding generations was checked only during the beginning of the standardizing procedures, and consequently only minimum numbers can be given.

Two different kinds of procedure were performed, viz. standardizing the X-chromosomes and making the autosomes homozygous.

*The X-chromosome.* — The X's were standardized by introducing the genes *sc cv v car*. The stock-cultures used to synthesize strains relevant to the present article were

$$\begin{array}{c} f B E b / + \\ sc cv v f \\ sc cv v f car \\ f B \\ f fu \end{array}$$

The males in some of the stocks (e. g., *f fu*) were kept going by crossing to *y* attached X females. The *sc cv v f car* stock had at an earlier instance been made by crossings between flies from the *sc cv v f* stock and a stock with *car*. The standardization procedure for the females was as follows:  $f B E b / + \times sc cv v f car$ ;  $f B E b / sc cv v f car \times sc cv v f car$ ;  $sc cv v f B E b / sc cv v f car \times sc cv v f car$ ;  $sc cv v f B E b car / sc cv v f car \times sc cv v f car$ .

Males *f B*:  $sc cv v f / sc cv v f \times f B$ ;  $sc cv v f / f B \times sc cv v f$ ;  $sc cv v f B / sc cv v f \times sc cv v f car$ ;  $sc cv v f B / sc cv v f car \times sc cv v f car$ ;  $sc cv v f B car / sc cv v f car \times sc cv v f B car$ ; *y* attached X  $\times sc cv v f B car$  («series B»; vide infra).

Males *f fu*:  $sc cv v f car / sc cv v f car \times f fu$ ;  $sc cv v f car / f fu \times sc cv v f car$ ;  $sc cv v f car / sc cv v f car \times sc cv v f fu$ ;  $sc cv v f car / sc cv v f$

$fu \times +$  (from strain »hom.+»; vide infra);  $y$  attached  $X \times sc cv v f fu$  car (»series B»; vide infra).

*The autosomes.* — The autosomes were made homozygous by means of a number of inbreeding procedures (Fig. 1).

First a wild type strain was propagated by brother—sister matings in pair-cultures. This strain was called »hom.+». Secondly  $y$  females with attached  $X$ 's were crossed with males from hom.+ of the 12th (or perhaps of a still later) inbreeding generation. This constitutes

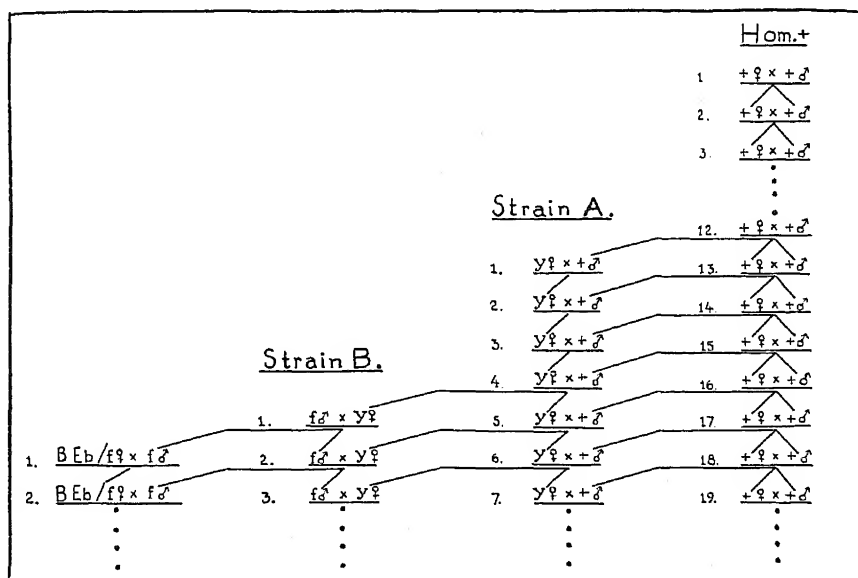


Fig. 1. Pedigree of lines Hom.+ , Strain A, Strain B and  $B Eb$  (cf. text).

»series A». In every new generation of A,  $y$  females were crossed to +males from later generations of series hom.+ . Simultaneously  $y$  females from series A were crossed to  $X$ -chromosome standardized males with  $f$ ,  $f B$ , and  $f fu$ . These series constitute the different »series B». In the case of  $f$  males, for instance, these were crossed to  $y$  females of the fifth generation of series A, and  $f$  sons (generation 2 of »series B with  $f$ ») were crossed to  $y$  females of a new generation of series A. It was not always possible to keep the generations of series A and series B exactly timed, with the effect that the generation number of the males from »series B with  $f$ » did not remain exactly 5 short of the generation number of the females taken from series A during the whole procedure. Parallel with these crosses females  $f B Eb / f$  (with standardized  $X$ 's)

were crossed in the same way with males of the »series B with  $f$ ». The first generation of females  $f B Eb/f$  was crossed with  $f$  females of this B series of generation 2. Fig. 1 shows how the different crosses were chained to each other. Series B with  $f B$  and with  $f fu$  were also made up in the same way as »series B with  $f$ ».

The following data give an idea of the number of generations within this procedure of making the autosomes homozygous.

The first generation of  $y$  attached females, series A, was crossed with wild males of the 12th (or perhaps a still later) generation of hom.+.

»Series B with  $f$ » was begun by crossing an  $f$  ( $X$  standardized) male with a  $y$  attached  $X$  female from the 5th generation of series A. The last cross in this series was made on Sept. 18, 1942, by crossing a  $y$  attached  $X$  female of the 23rd generation of series A with an  $f$  male of the 15th generation of »series B with  $f$ ». Thereafter the chain was broken and the crosses  $y$  attached  $X \times f$  ( $X$  standardized) were performed by inbreeding. Usually 2 females and 3 males from a single culture bottle were used as parents for the next generation. No check was then made of the number of these inbreeding generations.

Females  $f B Eb/f$  of the first generation were crossed with males  $f$  ( $X$  standardized) of the 2nd generation of »series B with  $f$ ». The last of these crosses was made on Sept. 26, 1942, with females of the 15th generation. Thereafter inbreeding was as in the above case.

»Series B with  $f B$ » was started by crossing an  $f B$  ( $X$  standardized) male with a  $y$  attached  $X$  female of the 6th generation of series A. The last cross was made on Sept. 24, 1942, between a female of the 23rd generation from series A and a male of the 14th generation of the »series B with  $f B$ ». Thereafter inbreeding as in the above cases.

»Series B with  $f fu$ » was started by crossing an  $f fu$  ( $X$  standardized) male with a  $y$  attached  $X$  female of the 22nd generation of series A. The last cross was made on Sept. 29, 1942, between a female of the 24th generation of series A and a male of »series B with  $f fu$ » of the 3rd generation. Thereafter inbreeding as in the above cases. After breaking the chains (which was necessitated by shortage of staff; see Fig. 1) the different lines have been propagated by more or less close inbreeding. (In the series hom.+ , for instance, this inbreeding has been performed as brother—sister matings and has now — Oct. 1946 — passed its 126th generation.)

When these preliminaries had been fulfilled, an  $f B Eb/f$  female was crossed with an  $f fu$  male, and thereafter  $f B Eb/f fu$  females were

mated generation after generation with *f fu* males by breeding two females to three males from a single culture bottle of the preceding generation. In this new line of crosses, which hereafter will be named the *original f B Eb/f fu* line, search was made for cross-over chromosomes carrying *Eb* but not *B*. Non-cross-over females have either round eyes and are forked and fused, or they have Bar eyes of the narrow type and are forked but not fused. Females with round eyes showing forked but not fused must be cross-overs between *B* and *fu*, and such females were accordingly tested by outcrossing with *f B* males (from »series B with *f B*»). If the females of the progeny had the ordinary heterozygous

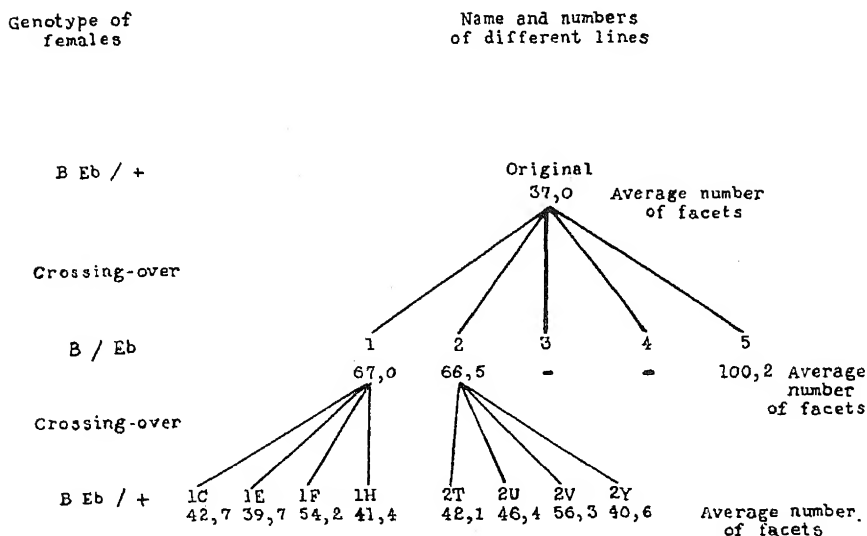


Fig. 2. Genealogy of the lines studied in the present paper. The genotypes of the females as given in the diagram show only the genes *B* and *Eb*. Apart from these genes a number of other genes were included in the genotypes (see text). The lines 1 C, 1 E, 1 F, 1 H, 2 T, 2 U, 2 V and 2 Y are in the text named »series», whereas the series 1 C, 1 E, 1 F, 1 H taken together, and the series 2 T, 2 U, 2 V, 2 Y taken together form two »groups», viz. group 1 and group 2. The line »original» is also in certain comparisons reckoned as a group.

Bar appearance, their mother could not be a carrier of *Eb*, and these cultures were consequently discarded. But if half of this female progeny showed the narrow Bar type the mother must have been an *f Eb/f fu* female. On five different occasions such females were found, and from each of these females a new line was started by the same kind of inbreeding and by alternating the crosses *f Eb/f fu* × *f B* and *f Eb/f B* × *f fu*. Before any facet counts were made, two of these lines

(viz. lines 3 and 4) were lost. Moreover, within these lines cross-overs between *B* and *Eb* were looked for in the progenies of the crosses  $f Eb/f B \times f fu$ . Forked daughters which showed the narrow Bar condition, thus being of the constitution  $f B Eb/f fu$ , were crossed with  $f fu$  males, and this was repeated for each generation. Within strain 1 such cross-overs occurred on four different occasions, giving rise to the strains 1 C, 1 E, 1 F and 1 H. Likewise line 2 gave rise in a similar way to the four strains 2 T, 2 U, 2 V and 2 Y. No cross-overs were found within the lines 3, 4 and 5. Fig. 2 shows the descent of the different lines.

The facet counts comprised two different sets of lines, viz. those where the females (disregarding all genes except *B* and *Eb*) were of the constitution *Eb/B* (lines 1, 2 and 5), and those where the females had the constitution *B Eb/+* (lines »original», 1 C, 1 E, 1 F, 1 H, 2 T, 2 U, 2 V, 2 Y). It was the intention of the standardization procedure to make all lines of *Eb/B* and *B Eb/+* respectively exactly isogeneous, apart from differences within the region from *B* to *Eb*. Thus before going further in our description we must first consider in greater detail how far this procedure can be assumed to have gone.

*The X-chromosome.* — From the above description of the standardization of the X-chromosome it will be found that the »original» line of females *B Eb/+* might have been heterozygous for some parts within the region from *f* to *car*. But since the procedure by which the lines 1, 2, 3, 4 and 5, and from them the lines 1 C, 1 E, 1 F, 1 H, 2 T, 2 U, 2 V, 2 Y have been established, consists of crossing-over between *B* and *Eb*, all lines may be looked upon as quite isogeneous apart from differences within the *B—Eb* region.

*The autosomes.* — It will be found in the above description that the strain (Fig. 2) by which the »series B with *f fu*» was established had to be broken and substituted by inbreeding rather too early. In consequence, it cannot be claimed with certainty that »series B with *f fu*» was homozygous from the beginning. Neither can it be definitely claimed that its autosomes after further inbreeding were made isogeneous to the autosomes of »series B with *f B*», it being very much more certain that the latter series was already homozygous when first used. The first female of the »original» line — i. e. the female in the first cross  $f B Eb/f fu \times f fu$  — may thus to a certain degree have been heterozygous in its autosomes. But the number of inbreeding generations (unfortunately not counted) which elapsed before the line 1 was established through crossing-over between *B* and *Eb* was certainly not

less than 20. However, as described above, the females of the lines 1, 2, 3, 4 and 5 were alternatively crossed to  $fB$  and  $f fu$  males, and since the autosomes of these two male lines may, as pointed out above, have been somewhat non-isogeneous, new heterozygosities may have been introduced. But there elapsed again a minimum of 20 generations from the establishment of line 1 to the establishment of lines 1 C, 1 E, 1 F and 1 H, and the same was the case from the establishment of line 2 to the establishment of the lines 2 T, 2 U, 2 V and 2 Y. Thus, though it is known that the speed with which the homozygotisation proceeds varies considerably in different lines (cf. BONNIER, 1947), it may be claimed reasonably and with a high degree of probability that at least the four lines 1 C, 1 E, 1 F, 1 H *inter se*, and the four lines 2 T, 2 U, 2 V, 2 Y *inter se*, were isogeneous — disregarding of course the differences within the  $B-Eb$  region.

### III. ANALYSIS OF FACET COUNTS.

The different lines in which facet counts were to be made had been established at different instances. The facet counts were, however, made in cultures reared simultaneously, and therefore the number of generations which elapsed from the first establishment of a certain line until facet counts, were different for different lines. According to the working plan, it was hoped that there would have been available at least 4 lines with  $B/Eb$  females, and from each of these at least 4 lines with  $B Eb/+$  females. But on account of the small percentage of crossing-over between  $B$  and  $Eb$  this hope was never fulfilled. And as time passed, it was decided to perform the facet counts with the lines which were available. From one single culture bottle of each of the inbred lines a number of pair cultures were made up, and all these culture bottles from all the different lines were raised simultaneously in one single incubator at 25° C. In order to avoid overcrowding all bottles contained abundant food. Any other measures for the standardization of the environmental conditions, for instance as proposed by CHEVAIS (1942), were not taken. In order not to interfere with the temperature the incubator was kept closed until the 16th day after the start, when the cultures were emptied and all females preserved in alcohol. The left eyes were dissected and the counts were made by the same technique (slightly modified) as described in the earlier paper (BONNIER, NORDENSKIÖLD and BÅGMAN, 1943). In Table 2 are the results summarized for all females of the constitution  $B Eb/+$ ,

TABLE 2. *Facet counts of eyes from all flies of the constitution B Eb/+.*  
*The standard errors are computed by dividing the average mean square within cultures 28,289 (see Table 4) by the number n and taking the square root, i. e. the number  $\frac{5,3188}{\sqrt{n}}$ .*

«Group»	«Series»	Cul- ture	Number of left female eyes counted (n)	Average facet number	»Group»	»Series»	Cul- ture	Number of left female eyes counted (n)	Average facet number
Original		1	23	39,83 ± 1,11	2	2 T	1	56	40,89 ± 0,71
		2	31	39,81 ± 0,96			2	51	44,18 ± 0,74
		3	21	40,10 ± 1,16			3	64	41,09 ± 0,66
		4	9	38,67 ± 1,77			4	66	44,42 ± 0,65
		5	8	38,75 ± 1,88			5	27	40,63 ± 1,02
		6	40	30,70 ± 0,84			6	17	42,71 ± 1,29
							7	35	44,97 ± 0,90
	Total	132	36,96 ± 0,46	8			56	37,89 ± 0,71	
1	1 C	1	37	48,27 ± 0,87			9	39	42,67 ± 0,85
		2	45	44,24 ± 0,79			Total	411	42,06 ± 0,26
		3	26	43,54 ± 1,04		2 U	1	52	45,67 ± 0,74
		4	33	45,30 ± 0,93			2	31	45,36 ± 0,96
		5	54	38,46 ± 0,72			3	54	46,57 ± 0,72
		6	28	40,36 ± 1,01			4	54	49,78 ± 0,72
		7	40	51,55 ± 0,84			5	14	47,43 ± 1,42
		8	70	40,71 ± 0,64			6	29	41,95 ± 0,90
		9	39	34,44 ± 0,85			7	44	47,91 ± 0,80
		Total	372	42,62 ± 0,28			8	46	42,22 ± 0,78
	1 E	1	53	40,51 ± 0,73			9	54	49,04 ± 0,72
		2	45	39,19 ± 0,79			Total	378	46,43 ± 0,29
		3	42	42,24 ± 0,82		2 V	1	17	52,71 ± 1,29
		4	39	37,23 ± 0,85			2	7	57,29 ± 2,01
		5	57	37,53 ± 0,70			3	11	56,64 ± 1,60
		6	56	42,48 ± 0,71			4	21	57,33 ± 1,16
		7	38	38,15 ± 0,86			5	11	55,35 ± 1,60
		8	57	38,93 ± 0,70			6	19	63,37 ± 1,22
			Total	387			39,66 ± 0,27	7	10
	1 F	1	7	54,29 ± 2,01			8	22	53,05 ± 1,13
		2	4	56,25 ± 2,06				Total	118
		3	8	53,13 ± 1,88		2 Y	1	7	38,00 ± 2,01
			Total	19			51,21 ± 1,22	2	6
	1 H	1	10	35,20 ± 1,68			3	30	38,70 ± 0,97
		2	44	42,52 ± 0,80	4		40	40,95 ± 0,84	
		3	50	40,30 ± 0,75	5		5	43,00 ± 2,38	
		4	25	36,80 ± 1,05	6		47	41,04 ± 0,78	
		5	54	44,35 ± 0,72	7		43	41,72 ± 0,81	
		6	53	42,09 ± 0,73			Total	178	40,62 ± 0,40
		7	44	40,27 ± 0,80	Total 2			1085	44,90 ± 0,16
		8	33	42,21 ± 0,93			2308	42,80 ± 0,11	
		Total	313	41,37 ± 0,30	Total				
Total 1			1091	41,42 ± 0,10					

TABLE 3. *Facet counts of eyes from all flies of the constitution B/Eb. The standard errors are computed by dividing the average mean square within cultures, 106,3514, by the number n and taking the square root, i. e. the number  $\frac{10,3127}{\sqrt{n}}$ .*

Line	Culture	Number of left female eyes counted (n)	Average facet number
1	1	58	77,29 ± 1,35
	2	65	70,92 ± 1,28
	3	17	57,29 ± 2,50
	4	48	75,94 ± 1,49
	5	55	63,95 ± 1,39
	6	54	65,56 ± 1,40
	7	49	79,08 ± 1,47
	8	72	64,08 ± 1,22
	9	28	70,00 ± 1,95
Total		446	70,00 ± 0,49
2	1	48	69,02 ± 1,49
	2	49	64,08 ± 1,47
	3	28	64,07 ± 1,95
	4	57	65,68 ± 1,37
	5	42	66,41 ± 1,59
	6	44	63,48 ± 1,55
	7	36	75,60 ± 1,72
	8	48	66,15 ± 1,49
	9	59	65,42 ± 1,34
Total		411	66,49 ± 0,51
5	1	20	105,15 ± 2,31
	2	10	91,80 ± 3,26
	3	25	105,28 ± 2,06
	4	19	92,58 ± 2,37
Total		74	100,16 ± 1,20
Total		931	70,84 ± 0,34

and in Table 3 for the females of the constitution B/Eb. The standard errors for the average facet number in each culture are computed from the average mean square within cultures by taking its square root and dividing by the square root of the number of eyes. Thus, if this number

in a certain culture is  $n$ , the standard error is  $\frac{5,3188}{\sqrt{n}}$  in Table 2, and  $\frac{10,3127}{\sqrt{n}}$  in Table 3.

*Females of the constitution B Eb/+.* — Beginning with the results shown in Table 2, the following nomenclature will be used. All cultures belonging to one and the same line of the lines 1 C, 1 E, 1 F, 1 H, 2 T, 2 U, 2 V or 2 Y will be named a »series»; the four series 1 C, 1 E, 1 F and 1 H form a »group», and the same is the case with the four series 2 T, 2 U, 2 V and 2 Y as well as with the »original» line.

A glance at Table 3 shows that there possibly is a correlation between the number,  $n$ , of eyes counted in the different cultures and the average facet number, there seemingly being less facets the greater the value of  $n$ . This effect, if real, is certainly of an environmental nature and must be due to differential crowding. Now the number  $n$  is not exactly the same as the number of females which hatched out in the different cultures, because of the fact that the facets were not recorded on every female. The number of females were never counted, but it can certainly be claimed that the number  $n$  is — if not exactly so, at

TABLE 4. *Analysis of variance of the whole female material B Eb/+.*

Source of variation	Degrees of freedom	Mean squares
Between groups of cultures of different size .....	5	2193,8251
Within groups of cultures of equal size		
Between groups .....	8	1274,8670
Within groups		
Between series .....	18	1351,5746
Within series		
Between cultures .....	35	400,8894
Within cultures (error) .....	2241	28,2899
T o t a l	2307	53,2835

$$\text{Ratios } \frac{2193,8251}{1274,8670} = 1,72; \quad P > 0,2$$

$$\frac{1351,5746}{400,8894} = 3,37; \quad P < 0,001$$

$$\frac{400,8894}{28,2899} = 14,17; \quad P < 0,001$$

TABLE 5. *Analysis of variance of females B Eb/+ belonging to »group» 1.*

S o u r c e o f v a r i a t i o n	Degrees of freedom	Mean squares	Symbols for reference to Table 7
Between groups of cultures of different size	4	449,6775	
Within groups of cultures of equal size .....		38,7113	<i>B</i>
Between series .....	8	848,6048	
Within series .....		32,7614	<i>b</i>
Between cultures .....	15	529,7725	
Within cultures .....	1063	25,7481	<i>w</i>
T o t a l	1090	40,2792	<i>t</i>

$$\text{Ratio } \frac{848,6048}{529,7725} = 1,60; \quad P = 0,2$$

TABLE 6. *Analysis of variance of females B Eb/+ belonging to »group» 2.*

S o u r c e o f v a r i a t i o n	Degrees of freedom	Mean squares	Symbols for reference to Table 7
Between groups of cultures of different size	5	1439,2861	
Within groups of cultures of equal size.....		52,6565	<i>B</i>
Between series .....	10	1762,0617	
Within series .....		36,6659	<i>b</i>
Between cultures .....	17	267,8997	
Within cultures .....	1052	32,9292	<i>w</i>
T o t a l	1084	59,0524	<i>t</i>

$$\text{Ratio } \frac{1762,0617}{267,8997} = 6,58; \quad P < 0,001$$

least with a very good approximation — proportional to the number of females hatched out. In the statistical analysis, therefore, in order to avoid the differential influence of the order of magnitude of *n* the cultures have first been grouped according to the value of *n* in 6 differ-

ent groups, viz.  $n = 4-10, 11-20, 21-30, 31-40, 41-50, 51-70$ . All comparisons have thereafter been made *within* these groups of  $n$ -values. The results of the computations are put together in Table 4, where the whole material of  $B\ Eb/+$  eyes from Table 2 is analysed, and in Tables 5 and 6 where the results from groups 1 and 2 are dealt with separately. From these three tables a number of conclusions may be drawn.

(1) The mean square in Table 5 (group 1) corresponding to the four series averages is statistically not very much larger than the mean square corresponding to culture averages ( $P > 0.2$ ). But in Table 6 (group 2) the first of these mean squares is significantly very much larger than the latter, and gives a mean square ratio of 6.58 which corresponds to  $P$  less than 0.001. This is by far the most interesting of the facts ascertained from Tables 4, 5 and 6. It might be objected that in group 2 (Table 6) there is an unusually small variation between the cultures, the mean square being only about 268, whereas in group 1 (Table 5) it is about 530. But even when the mean square between series from group 2 (Table 6), 1762.0617, is compared with the average mean square between cultures from groups 1 and 2, which is 390.6526 (not explicitly shown in the tables), a mean square ratio of 4.51 is found, and this (with 32 degrees of freedom) corresponds also to a  $P$  which is somewhat less than 0.001. Now, the four series within group 2 (as within group 1) were according to the preceding paragraph very probably isogeneous, apart from differences within the  $B-Eb$  region. The conclusion is that there must be genetical differences between the series belonging to group 2, and, granting the existence of the isogeneity, it is just those differences within this region which must be assumed responsible for the differences in facet numbers between the series means of group 2.

(2) As the mean square corresponding to group averages *within*  $n$ -values (Table 4) is only insignificantly less, and as the mean squares corresponding to series averages *within*  $n$ -values (Tables 5 and 6) are larger than the mean squares corresponding to the variations *between* different  $n$ -values, it is possible that the partition of the material according to the orders of magnitude of  $n$  was unnecessary. On the other hand, such a partition, even if it is not necessary, can cause no harm and will in no way interfere with the other conclusions to be made.

(3) As the mean squares corresponding to group averages (Table 4) is smaller than the mean square corresponding to series averages, the facet number averages of the three groups are statistically equal. The

same conclusion would have been drawn if only the two groups 1 and 2 had been compared.

(4) Within all series the mean squares corresponding to culture averages are very much larger than the mean squares corresponding to the variation within cultures, and consequently the environmental conditions prevailing within different culture bottles must have been effectively different in spite of the simultaneity of the raising of all cultures.

In this connection attention should be called to the fact that the partition of the material with respect to order of magnitude of  $n$  lowers the available degrees of freedom. The reason will be found obvious when it is mentioned that, for instance, for  $n$  lying between 11—20 only one group (group 2) is represented, and for  $n$  lying between 31—40 only one culture is represented within the series 1 H. It follows that if no such partition with respect to the  $n$ -value had been performed, not only would changed degrees of freedom have been found, but also changed significance values  $P$ . In this case the ratio between the mean squares corresponding to series and culture averages (within the series) had been, in the case of groups 1 and 2, 3,<sub>34</sub> and 3,<sub>39</sub> respectively. The corresponding degrees of freedom are: group 1, 3 and 24; group 2, 3 and 29 and thus for both groups  $P$  lies between 0,<sub>05</sub> and 0,<sub>01</sub>. If both groups are pooled the ratio is 13,<sub>47</sub> and  $P$  is less than 0,<sub>001</sub>. Thus whether the partition in question is performed or not, it must be concluded that statistically significant differences between means of series belonging to one and the same group do exist.

TABLE 7. *Subdivision of the total variance in facet numbers in B Eb/+ flies according to different causes of variation.*

Cause of variation	Formula for calculation (see Tables 5 and 6)	Percentage of total variance	
		Group 1	Group 2
Influence of size of culture .....	$\frac{t-B}{t}$	4	11
Differences between »series» within »groups» of cultures of equal size .....	$\frac{B-b}{t}$	15	27
Differences between cultures of equal size belonging to the same »series» .....	$\frac{b-w}{t}$	17	6
Differences between flies that hatched out within the same culture bottle .....	$\frac{w}{t}$	64	56

As it may be of some interest to know to what extent the total variation in facet numbers is due to the different sources of variation, estimates are made in Table 7 on the basis of the figures in Tables 5 and 6. It is seen that in group 1, as well as in group 2, the largest part of the variation is due to causes within the culture bottles. These causes cannot be analysed further, and are therefore as usual lumped together under the name of error. The largeness of this variation seems, however, to make it certain that the environmental conditions within the different bottles have been effectively different. In the case of group 2, where we have found the differences in series means to be highly significant, these differences are responsible for a variation, which next to error, is the largest one.

*Females of the constitution B/Eb.* — The counts within the lines 1, 2 and 5 of the constitution *B/Eb* were given in Table 3. To make a very thorough statistical analysis of these counts is of less importance, as their isogeneity is less certain. We therefore confine ourselves to making the remark that the line 5 has a facet average which differs considerably from the averages for lines 1 and 2. We believe that part of the cause of this difference is to be found in differences within the *B—Eb* region, but on account of the uncertain isogeneity, autosomal differences cannot be excluded.

#### IV. DISCUSSION AND CONCLUSIONS.

It has been shown in the preceding paragraph that facet number averages from *B Eb/+* females belonging to different series within one and the same group are significantly different (at least in group 2). These differences cannot be assumed to be due to environmental causes. As has been pointed out, all cultures were raised simultaneously within the same incubator, and the environment can thus have a differential influence only in one of the following two ways: (1) on the individual fly within a certain culture bottle and/or (2) on the aggregate of the flies from the separate bottles. But in each case a »series» was made up of several bottles, and all bottles were mixed together in the incubator. It is thus extremely unlikely that environment can have influenced the series averages, and the differences between the series averages must be assumed to be due to genetical causes.

There are two possible kinds of genetical differences between the series within a group. (1) It may be remembered that each series originated from a single case of crossing-over between *B* and *Eb* in a

*B/Eb* female. The first kind of genetical difference, which must be assumed to occur, thus has its origin in different points of crossing-over within the *B—Eb* region at the moment the various series are founded. (2) The second cause is that in spite of the effects caused, the isogeneity of the different series — apart from the *B—Eb* region — was not complete, with the consequence that the series still differed with respect to genes affecting the facet number, but situated elsewhere.

As such efforts were taken to achieve isogeneity, it seems very improbable that differences due to modifying genes should still be possible. There is also an additional piece of evidence which, in its own way, speaks in favour of the isogeneity. The group which we have called »original» was directly, i. e. without any crossing-over, derived from the line of *B Eb/+* females described in the preceding article (BONNIER, NORDENSKIÖLD and BÅGMAN, 1943), and which is denoted as strain No. 2 in Table 1 of the present article. In this strain 190 eyes were counted and gave an average of 40,5 facets per eye. This average should therefore, if no further changes due to non-isogeneity had occurred, agree with the average of the »original» (Table 2), which is 37,0. An analysis of variance shows that the difference between the two averages 40,5 and 37,0 is insignificant. By making this analysis we cannot carry out a partition with regard to culture size, as no such partition was performed when the facets of the 190 eyes, strain 2 (Table 1), were counted. The mean square between the average of strain 2 (Table 1) and average of »original» (Table 2) is 1020,1925, and the mean square between the culture averages in »original» is 453,1750. The ratio of these two mean squares is 2,25, which, with the degrees of freedom 1 and 5 respectively, corresponds to a value for P not much less than 0,2. Now, as is seen from Table 2, »original» is made up of 6 cultures of which the last one differs considerably from the first 5. To make the same kind of analysis of variance after exclusion of the last culture would, however, not be fair, as those first 5 cultures are unusually uniform (as compared with the cultures within the other series). It seems most correct to compare the above mentioned mean square 1020,1925 with the mean square between averages of cultures belonging to one and the same series, and calculated from the whole material of Table 2. This last mean square is 739,8446, and the ratio of the two mean squares is 1,38, which, with the degrees of freedom 1 and 58, corresponds to a value of  $P > 0,2$ . It may finally be added that between the dates of the hatching out of the females of strain 2

(Table 1) and the females of »original» (Table 2) there had elapsed at least 75 generations.

It can never be stated if and when homozygosity is complete after a limited number of inbreeding generations even if this number is very large. Although it is thus impossible to prove decisively that the place of crossing-over is the *only* genetical determining factor in the variation of facet number in different »series» belonging to the same »group» it has, however, been proved as highly probable. Now, as mentioned above, *Eb* occurred in a Bar chromosome. But an *Eb* non-Bar chromosome contains only one of the two Bar segments. Furthermore, *Eb* has, when Bar is present, an effect of the same kind as Bar. From these considerations it was tentatively proposed in the preceding article (BONNIER, NORDENSKIÖLD and BÄGMAN, 1943) that *Eb* is in itself a part of Bar. In this article it was also pointed out that the fact that, by ordinary linkage tests, *Eb* is found to be situated to the right of Bar, may be explained by supposing that *Eb* lies within the Bar segment but so close to its right border that crossing-over between *Eb* and this right border has never occurred. But as crossing-over in different points within the *B—Eb* region gives different results, with regard to facet number, we must conclude that *Eb* has a linear dimension and that it is consequently not a »point» gene, but occupies a certain portion of the chromosome, which conforms with the crossing-over reducing effect of *Eb*. If this portion is not too short it is conceivable that one could find crossing-over cases giving the inverted sequence *Eb B* instead of the sequence *B Eb*. Concerning the position effect of *Eb*, it should indicate that the functioning of the *Eb* portion of the chromosome and its final effect on facet number depends upon how much of this portion lies in the one, and how much in the other *X*-chromosome. Thus, if the *Eb* portion is made up of two parts *Eb'* and *Eb''*, both genotypes *B Eb/+* and *B/Eb* should more correctly be symbolized as *B Eb'/Eb''*. And the phenotype would then be dependent upon the relative lengths of the two parts *Eb'* and *Eb''*.

In this connection it is worth recalling that SUTTON (1943), in her studies on translocations within the Bar region, found Bar effects of different strengths. The phenotypic effects of her different translocations thus showed themselves as different numbers of facets.

As an alternative to the supposition that *Eb* is divided in portions *Eb'* and *Eb''*, it may be thought that it is the gene Bar itself which has been divided differently in the different series of our experiment. If this is so, it would be possible to find similar results in experiments with

Bar even in cases when *Eb* is not present. In new experiments, therefore, an attempt will be made to determine whether changes from *BB* to *B* and from *B* back to *BB* will lead to different numbers of facets in different series. But as the great obstacle to making conclusive proofs is the impossibility of being quite certain of the isogeneity when this is striven after by a procedure of homozygotisation, an attempt will be made to preserve isogeneity in these experiments by permanent heterozygosity.

Granting that the results which we have described in the present paper are not due to non-isogeneity, a certain kind of »gene divisibility» has been demonstrated. The possibility of such a divisibility has been discussed earlier in connection with the differential effects of scute inversions by RAFFEL and MULLER (1940). Our findings may possibly also support GOLDSCHMIDT's theories on the chromosome and the hereditary units (cf. GOLDSCHMIDT, 1946).

### SUMMARY.

(1) In a preceding article (BONNIER, NORDENSKIÖLD and BÅGMAN, 1943) the sex-linked lethal gene »Exaggeration of Bar» with the symbol *Eb* was described. This gene occurred spontaneously in a Bar chromosome, but the *Eb* chromosome has only one of the duplicated Bar segments. By ordinary linkage tests *Eb* was localized as lying very close to the right of Bar. It reduces crossing-over between *B* and *fu* from 2.5 to about 1.5 units. A female heterozygous with respect to *Eb*, but not containing *B*, has round eyes; but when *B* is present *Eb* has the effect of a *B* gene. Thus, for instance, a female heterozygous with respect both to *B* and *Eb* has eyes of the homozygous Bar type. *Eb* shows also the typical Bar position effect inasmuch as *B Eb/+* females have narrower eyes than *B/Eb* females. It was also tentatively proposed that *Eb* is situated within the Bar region.

(2) From a strain of *B Eb/+* females, in the present article named »original», *B/Eb* females occurred through crossings-over on 5 different occasions, and, from each of these, strains of *B/Eb* females were established. (Later, however, two of these were lost.) From two of these strains again *B Eb/+* females occurred through crossings-over at four different instances in each of the two *B/Eb* strains. In this way 2 »groups» of *B Eb/+* strains were established, each containing 4 »series» (see Fig. 2). From all the different strains a number of cultures were raised simultaneously in the same incubator at 25° C, and the

number of facets in the left eyes of the female progeny were recorded.

(3) Prior to this facet counting an effort was made to make all *B Eb/+* strains isogeneous through a procedure of marking the X-chromosome by known recessives and by making the autosomes homozygous by inbreeding. Such isogeneity can never be conclusively proved, but the manner in which the different series were established shows very probably that at least all series belonging to one and the same group are isogeneous. It is also shown that there are statistically significant differences between the facet numbers of flies belonging to different series, and that these differences cannot be caused by differential effects of environment.

(4) It is concluded that *Eb* — or perhaps the gene *Bar* itself — is »divisible» into parts, and that it is the lengths and interactions of these parts which are responsible for the determination of the facet number.

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# EINE PERIKLINALCHIMÄRE IN DER GATTUNG SYRINGA

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(With a Summary in English)

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ALS ich vor einigen Jahren mit einer Untersuchung über verschiedene Periklinalchimären beschäftigt war, erhielt ich bei einer Gelegenheit Mitteilung über einen Fliederstrauch, der einst einen Zweig mit ganz abweichender Blütenfarbe getragen hatte. Dieser Zweig war wieder verschwunden und da ich an anderen Exemplaren derselben Flidersorte keine abweichenden Blüten finden konnte, hielt ich es für wahrscheinlich, dass es sich um eine Farbenmutation gehandelt hatte. Später gefundene Literaturangaben, besonders von ALEXANDER BRAUN (1873, 1874), der seinerzeit diese Flidersorte eingehend studierte, wie auch weitere eigene Beobachtungen haben jedoch meine Ansicht ganz geändert; ohne Zweifel handelt es sich hier um eine Periklinalchimäre von interessanter Zusammensetzung.

Die *Syringa*-Form, um die es sich handelt, ist eine alte, heutzutage nicht oft angepflanzte Sorte, die unter dem Namen *Syringa chinensis* WILLD. var. *alba* (KIRCHN.) REHD. geht. In der Blattform stimmt sie mit *Syringa chinensis* überein. Die Blüten sind von abweichender Farbe; sie sind indessen nicht ganz weiss, wie der Name vermuten liesse, sondern blass lilafarbig; besonders an der Innenseite der Kronröhre und um ihre Mündung herum tritt der lila Anflug zutage; die Lappen des Saumes sind sehr schwach gefärbt, beinahe weiss. Interessant ist aber, dass die Blüten auch in der Form von *Syringa chinensis* abweichen und bestimmte Ähnlichkeiten mit *S. vulgaris* aufweisen, wie schon von BRAUN hervorgehoben ist. Die Lappen des Saumes sind nicht so ausgebreitet wie bei *S. chinensis*, sondern haben eingerollte Ränder, wie dies bei *S. vulgaris* der Fall ist, sodass sie schmaler und oben abgerundet aussehen, während sie bei *S. chinensis* im Verhältnis zur Länge breiter und kurz gespitzt erscheinen (siehe Fig. 1). Der Kelch, der bei *S. chinensis* rötlich ist und ziemlich lange Zähne hat, die durch spitzwinklige Einschnitte getrennt sind, ist hier wie bei *S. vulgaris* grün und hat breite, abgerundete Einkerbungen zwischen den relativ kurzen

Zähnen. Eine andere Ähnlichkeit mit *S. vulgaris* besteht darin, dass die Blütenrispen nicht so überhängend wie bei *S. chinensis*, sondern mehr aufrecht sind.

Bei dieser Fliedersorte sind nun bei verschiedenen Gelegenheiten Zweige oder kleinere Partien von abweichendem Aussehen beobachtet worden. Schon ALEXANDER BRAUN (1873, 1874) erwähnt einige solche Fälle. Er hat an einem Exemplar in Berlin in zwei aufeinanderfolgenden Jahren Blüten gefunden, die nicht nur die lila Farbe von *S. chinensis* hatten, sondern auch der Form nach ganz mit dieser übereinstimmten. Sie kamen entweder in besonderen Rispen vor, wo alle Blüten vom *chinensis*-Typus waren, oder auch es konnte ein Blütenstand sektorial

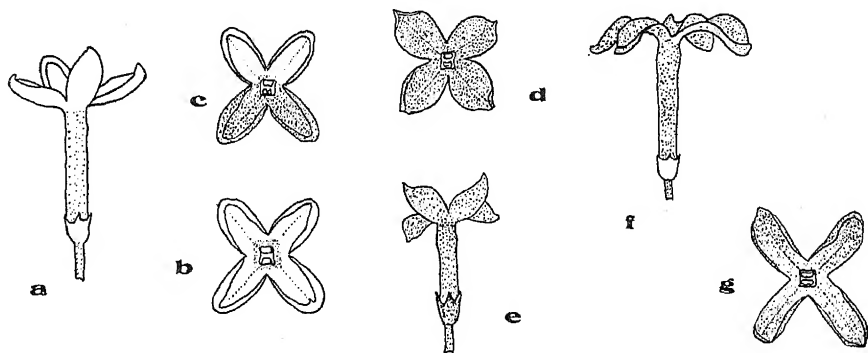


Fig. 1. Zwei Blüten der sog. *Syringa chinensis* var. *alba* (= *S. correlata*) im Botanischen Garten zu Lund (a—b), eine Blüte desselben Exemplares, die zur Hälfte lilafarbig ist (c), zwei *chinensis*-Blüten eines Rückschlagzweiges desselben Exemplares (d—e), zwei Blüten von *S. vulgaris* (f—g). — Schwach vergrößert ( $\times 1\frac{2}{5}$ ).

aufgeteilt sein, sodass ein Sektor vom gewöhnlichen Typus, ein anderer vom *chinensis*-Typus war und bestimmte Blüten sektorial in lila und blassfarbige Partien vom *chinensis*-, bzw. *alba*-Typus aufgeteilt waren. Ähnliche Abweichungen — vereinzelte Blüten oder auch Gesamtinfloreszenzen vom *chinensis*-Typus — werden u. a. von SCHÜBELER (1888) erwähnt, der sie mehrmals an in Norwegen gezogenen Exemplaren gefunden hat, und ferner von L. HENRY (1901), der in Frankreich in 7 verschiedenen Jahren an mehreren Exemplaren der Sorte von verschiedener Herkunft solche Abweichungen beobachtet hat.

An einem alten Exemplar der Sorte im Botanischen Garten zu Lund, das den gewöhnlichen Namen *S. chinensis* var. *alba* trägt, traten i. J. 1946 auch einige Abweichungen auf. An einem grossen Ast gab es in grosser Höhe, unweit des Gipfels, ein Zweiglein mit zwei Blüten-

rispen, die sowohl in der Farbe wie in der Gestalt der Blüten ganz mit *S. chinensis* übereinstimmten. Die Blüten waren nur etwas kleiner als bei *S. chinensis* gewöhnlich der Fall ist, aber auch die anderen Blüten auf dem Ast, der die Abweichungen trug, waren etwas kleiner als am übrigen Strauch; offenbar war der Ast schlecht ernährt oder aus anderer Ursache geschwächt. An einem anderen Ast kam in einer sonst typischen Blütenrispe eine vereinzelte Blüte vor, die halb lilafarbig, halb blass wie der Typus war. Die Grenze verlief über zwei entgegengesetzte Einschnitte des Saumes; es waren also zwei Abschnitte ganz lila, zwei von der Farbe der Varietät. Die Kronröhre ist ja auch bei dieser etwas gefärbt; man konnte aber deutlich sehen, dass genau die eine Längshälfte stärker gefärbt und scharf vom übrigen Teil abgegrenzt war; sie hatte übrigens einen hellen Mittelstreifen. Die diesem stärker gefärbten Kronenteil entsprechende Partie des Kelches war nur an der einen Seite in einem schmalen Band, ungefähr einem halben Kelchzahn entsprechend, vom *chinensis*-Typus; im übrigen stimmte der Kelch mit der Varietät überein. Der Gestalt nach wichen die lila Abschnitte dieser sektorial geteilten Blüte nicht merkbar von *S. chinensis* var. *alba* ab; sie waren etwas länger als die »weissen« Abschnitte, aber sonst hatten sie ungefähr dieselbe Form wie diese, mit ziemlich stark eingerollten Rändern. Dass sie nicht *chinensis*-Form hatten, ist vielleicht darauf zurückzuführen, dass die Aussenseite der Krone, wie der Kelch, möglicherweise zum grösseren Teil nicht vom *chinensis*-Typus war.

An einem Exemplar der Sorte in den städtischen Anlagen in Lund wurde i. J. 1946 ein grosser, kräftiger Ast von *S. chinensis* beobachtet; er zweigte indessen so tief unten am Stamm ab, dass man mit der Möglichkeit rechnen muss, dass er in der Unterlage, auf der der Strauch veredelt ist, seinen Ursprung hat; es braucht nicht unbedingt ein Rückschlag zu sein. Jedenfalls kommen Rückschläge zu *S. chinensis* nicht nur in seltenen Ausnahmefällen, sondern, wie besonders die Literaturangaben zeigen, ziemlich häufig bei der Sorte vor.

Als A. BRAUN seine Beobachtungen über die eigentümlichen Variationen bei *S. chinensis* var. *alba* — von ihm *S. correlata* genannt — vorlegte, führte er einige verschiedene Möglichkeiten zu ihrer Erklärung an, konnte aber keine ganz befriedigende Lösung des Rätsels finden — ganz natürlich in einer Zeit, wo die Natur der Periklinalchimären noch nicht klargelegt war. Heutzutage bietet es keine so grossen Schwierigkeiten, die Bildung der Form und die Ursachen ihrer vegetativen Aufspaltung festzustellen. Ohne Zweifel handelt es sich um eine Periklinal-

chimäre; dafür sprechen die hin und wieder erscheinenden Rückschläge zu *S. chinensis*, die oft bestimmte Sektoren in einer für Rückschläge der Periklinalchimären typischen Weise bilden, wie dies z. B. bei *Rhododendron Simsii* var. *Vervaeenaeum* und *Crataegus media* var. *punicea* (HJELMQVIST, 1944) vorkommt. Die eine Komponente der Chimäre besteht, wie die Rückschläge zeigen, aus *Syringa chinensis*, mit typischer, lila Blütenfarbe. Die andere Komponente ist nicht auf Grund von Rückschlägen bekannt, lässt sich aber mit Hinblick auf das Aussehen der Blüten als eine Form von *S. vulgaris* mit weissen Blüten erwarten. Dass es sich um eine von *S. vulgaris* und *chinensis* gebildete Periklinalchimäre handelt, wird durch einige morphologische und anatomische Merkmale bestätigt, die auch über die Anteile der beiden Komponenten an der Chimäre Aufschluss geben.

Was zuerst den Kelch und die Blütenstiele betrifft, so bestehen zwischen *S. chinensis* und *vulgaris* bestimmte Unterschiede in der Beschaffenheit der Oberfläche, also in der Ausbildung der Epidermis. Bei *S. chinensis* ist der Kelch, der wie erwähnt eine rötliche Oberfläche hat, von sitzenden Drüsen punktiert, bei *S. vulgaris* ist er grün und auf der Oberfläche und am Rand mit gestielten Drüsenhaaren versehen; solche Haare sind auch auf den Blütenstielen vorhanden, fehlen aber bei *S. chinensis* auch hier. Die var. *alba* stimmt in diesen Merkmalen vollkommen mit *S. vulgaris* überein: der Kelch ist grün und Drüsenhaare gibt es sowohl hier wie auch auf den Blütenstielen.

In der Anatomie der Blätter bestehen einige Unterschiede, die zwar nicht gross sind, aber doch gewisse Schlüsse gestatten. Die Epidermis hat in bezug auf die meisten Merkmale bei allen drei Formen ähnliches Aussehen; Spaltöffnungen kommen z. B. bei allen reichlich auf der Unterseite, spärlich auf der Oberseite vor; Drüsen kommen ebenso bei allen beiderseits vor. In Flächenschnitten von der Unterseite des Blattes (Fig. 2 a—c) ist indessen zu sehen, dass die Seitenwände der Epidermiszellen bei *S. chinensis* beinahe völlig gerade sind; bei *S. vulgaris* dagegen sind sie einigermassen gekrümmt, oft unregelmässig gewellt. Die Varietät *alba* stimmt hierin, wie betreffs der Epidermis des Kelches und der Blütenstiele, mit *S. vulgaris* überein.

Hinsichtlich des Inneren der Blätter (Fig. 2 d—f) bestehen keine scharf markierten Unterschiede. Bei *S. vulgaris* sind jedoch die zwei Palisadenschichten gewöhnlich von fast derselben Grösse; die Zellen der unteren Schicht sind nur wenig kleiner als die der oberen, annähernd ebenso lang; bei *S. chinensis* sind die Zellen der unteren Palisadenschicht gewöhnlich erheblich kleiner als die der oberen, oft nur halb so lang.

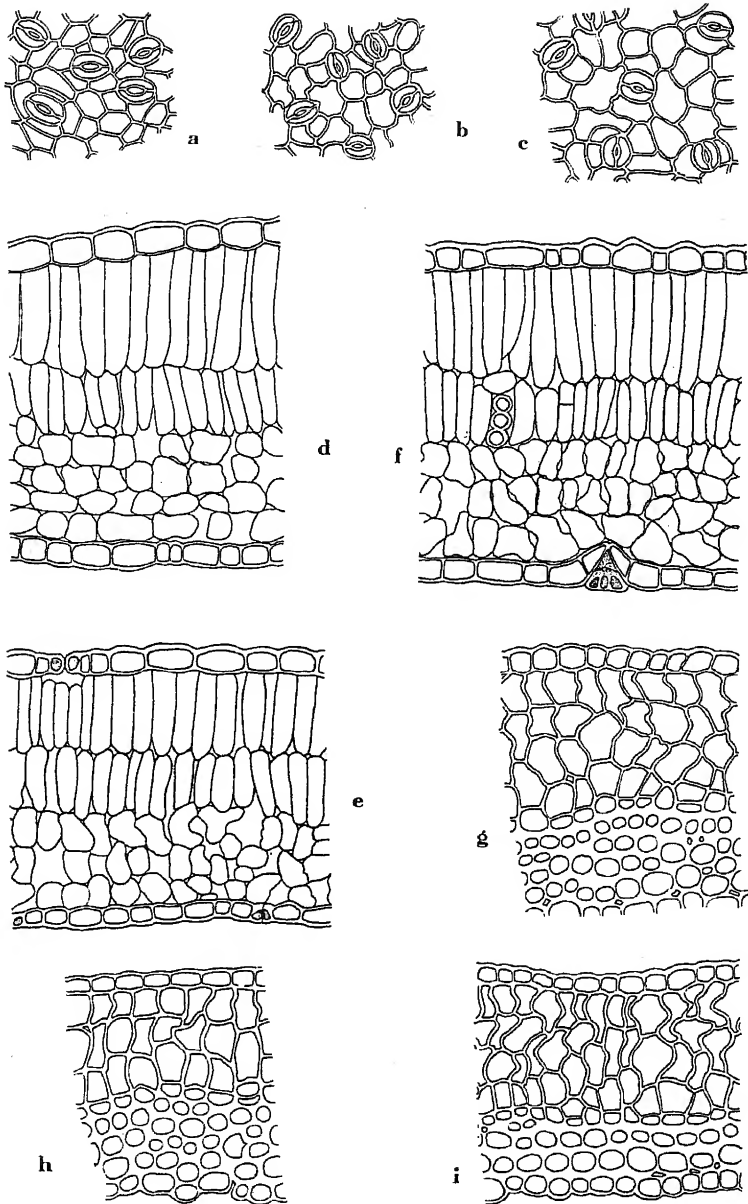


Fig. 2. Anatomische Unterschiede zwischen verschiedenen *Syringa*-Formen. — *a—c*: die Epidermis der Blattunterseite in Flächenansicht; *d—f*: Blattquerschnitte; *g—i*: Querschnitte durch den äusseren Teil einjähriger Zweige. — *a, f, g*: *S. chinensis*; *b, e, h*: *S. vulgaris*; *c, d, i*: die Chimäre. — Ca. 180fache Vergrösserung.

Dies ist auch bei var. *alba* der Fall, die also in diesem Merkmal mit *S. chinensis* übereinstimmt. Dieses Grössenverhältnis bildet zwar kein absolut unterscheidendes Merkmal — Variationen kommen auch an demselben Strauch vor —, aber wenn man nur Blätter unter denselben Umständen, Lichtverhältnissen usw. vergleicht und das vorherrschende Verhältnis im Auge behält, ist es doch möglich, einen Unterschied zu finden. Auch scheint das Schwammparenchym bei *S. chinensis* und var. *alba* etwas kompakter zu sein als bei *S. vulgaris*.

Auch in der Anatomie des Stammes kann man einige, wenn auch nicht grosse Unterschiede zwischen *Syringa vulgaris* und *S. chinensis* beobachten. In jüngeren Zweigen gibt es in der primären Rinde ein Kollenchymgewebe von ziemlich oberflächlicher Lage — nur durch ein ca. 3—4-schichtiges Parenchym von der Epidermis getrennt — und in diesem Kollenchym kann man vielleicht einige Unterschiede beobachten, wenn es auch bei jeder Form etwas variiert. Bei *S. vulgaris* (Fig. 2 h) sind die Zellen dieses Gewebes gewöhnlich ziemlich unregelmässig gelagert und mit ringsum ziemlich gleichmässig verdickten Wänden versehen. Das entsprechende Gewebe bei *S. chinensis* (Fig. 2 g) pflegt dagegen stark verdickte perikline Wände zu haben, während die antiklinen erheblich dünner sind, und die Zellen sind im allgemeinen regelmässig in konzentrischen Zellagen geordnet, die also durch starke konzentrische Membranleisten von einander getrennt werden. In derselben Weise verhält sich var. *alba* (Fig. 2 i). Im Mark des Stammes sind ferner die Zellwände bei *S. chinensis* und ebenso bei der var. *alba* fein und unregelmässig getüpfelt, während bei *S. vulgaris* von dieser Tüpfelung fast nichts zu sehen ist. Diese Unterschiede zwischen den beiden vorigen und der letzten Form kommen sowohl in einjährigen wie in älteren Zweigen und auch in den Infloreszenzachsen vor.

Betreffs verschiedener Organe stimmt also die Varietät *alba* im oberflächlichen Gewebe mit *S. vulgaris*, in inneren Geweben mit *S. chinensis* überein. Die Epidermis ist ohne Zweifel vom *vulgaris*-Typus; schon unmittelbar darunter scheint *chinensis*-Gewebe aufzutreten. Auf Grund der nicht allzu grossen Unterschiede zwischen *S. vulgaris* und *chinensis* kann vielleicht nicht behauptet werden, dass durch die Anatomie ganz bindend bewiesen ist, dass alle Zellagen unter der Epidermis von *S. chinensis* gebildet werden. Es geht auch nicht, durch Aufziehen von Samenpflanzen Auskunft über die Natur der Hypodermis zu gewinnen, denn die Form ist — wie im allgemeinen auch *S. chinensis* — steril und setzt keine Samen an. Es gibt indessen andere Tatsachen, die dafür sprechen, dass nur die Epidermis dem *vulgaris*-Typus angehört. Erstens ist ja

die Blattform ganz vom *chinensis*-Typus. Die Hauptmasse der Blätter geht ja aus der hypodermalen Zellage im Vegetationspunkt hervor; würde diese einer *vulgaris*-Form angehören, so würden die Blätter ein ganz anderes Aussehen bekommen; sie muss statt dessen zu *S. chinensis* gehören. Ferner ist von Untersuchungen des Vegetationspunktes bei *Syringa* durch A. SCHMIDT (1924) bekannt, dass hier in der Hypodermis nicht selten perikline Teilungen vorkommen. Aus diesem Grund ist SCHMIDT der Ansicht, dass eine diplochlamyde Chimäre bei *Syringa* kaum vorkommen, jedenfalls nicht Bestand haben kann. Es steht wenigstens fest, dass wenn eine solche entstehe, so würden Rückschläge zur äusseren Komponente sehr häufig vorkommen. Solche Rückschläge sind ja bei unsrer *Syringa*-Chimäre überhaupt nicht bekannt und sie kann darum nicht mehr als haplochlamyd sein.

Ich muss also zu dem Ergebnis kommen, dass *Syringa chinensis* var. *alba* eine Periklinalchimäre ist, bei der die unter der Epidermis liegenden Gewebe von der typischen *S. chinensis* mit lila Blüten gebildet werden, während die Epidermis von einer *S. vulgaris*-ähnlichen Form gebildet wird, die sich in keinem Rückschlag gezeigt hat, aber allem Anscheine nach die weissblütige *S. vulgaris* darstellt. Mit Rücksicht auf diese Zusammensetzung ist es ja nicht angemessen, die Form als Varietät zu *S. chinensis* zu rechnen, sondern gleichwie andere ähnlichen Chimären soll sie wohl einen eigenen Namen haben. Es scheint mir am richtigsten, den alten Namen A. BRAUNS *S. correlata* wieder einzuführen.

Die Eigenschaften, die *Syringa correlata* kennzeichnen, lassen sich leicht mit Hilfe der Annahme erklären, dass sie eine haplochlamyde Chimäre von der erwähnten Zusammensetzung ist. Das *chinensis*-ähnliche Aussehen der Blätter hat darin seinen Grund, dass *S. chinensis* die hypodermalen Zellagen bildet, aus denen die Blätter wie oben erwähnt zum grösseren Teil hervorgehen. Es wäre vielleicht zu erwarten, dass die Epidermis auch einen Einfluss auf die Blattform zu Ähnlichkeit mit *S. vulgaris* haben sollte, wie bei *Crataegomespilus Asnieri* und anderen haplochlamyden Chimären die Blattform auch von der äusseren Komponente bestimmt wird; dass dies nicht der Fall ist, wird jedoch dadurch erklärt, dass die Unterschiede in der Blattform zwischen *S. vulgaris* und *chinensis* sich auf Merkmale beziehen, die durch tiefere Zellagen bestimmt werden; sie beziehen sich ja meist auf die Gestaltung der Blattbasis. Dass die Blüte *vulgaris*-ähnlicher ist, findet darin seine natürliche Erklärung, dass Kelch und Blumenkrone, besonders ihre äusseren Partien, dünne Gebilde sind, die nur aus wenigen Zellagen bestehen, sodass die Epidermis dort eine relativ grössere Rolle spielt.

Es ist ja nicht unmöglich, dass die Randpartien wie bei gewissen anderen Blüten ganz von der Epidermis gebildet werden. Die Blütenfarbe ist wahrscheinlich durch miteinander konkurrierende Tendenzen in der Epidermis und den darunter liegenden Schichten zu erklären; diese haben Anlage für Anthocyanbildung in den Epidermiszellen; der Epidermis selbst fehlen vermutlich solche Anlagen gänzlich; dadurch erklärt sich, dass die dünnen Lappen des Saumes fast weiss werden, während die dickere Röhre etwas stärker gefärbt erscheint. Am meisten für die Farbe der Epidermiszellen entscheidend sind natürlich die eigenen Anlagen dieser Zellen; einigermassen können sie jedoch ganz sicher auch von den angrenzenden Zellagen beeinflusst werden, wahrscheinlich durch Zufuhr von Stoffen, die für die Anthocyanbildung nötig sind (vergl. *Rhododendron Simsii* var. *Vervaeaneanum* u. a.; HJELMQVIST, 1944).

Dass Rückschläge zu *S. chinensis* ziemlich häufig sind, während Rückschläge zur äusseren Komponente sehr selten sind, wenn sie überhaupt vorkommen, steht auch mit der haplochlamyden Natur der Chimäre im Einklang; bei haplochlamyden Chimären pflegen Rückschläge zur inneren Komponente erheblich häufiger aufzutreten als solche zur äusseren.

Über die Entstehung von *S. correlata* ist nichts Sicheres bekannt. Die ältesten Literaturangaben, die diese Form betreffen, dürften die Berichte A. BRAUNS (1873, 1874) über ein altes Exemplar von *S. correlata* im Berliner Botanischen Garten sein, das seinerzeit von einem Gärtner in Halle erhalten wurde. Nach Angabe war es auf *S. chinensis* gepfropft. Es erscheint möglich, dass die Form bei dieser Pfropfung entstanden ist, sodass die eingepfropfte Sorte eine *vulgaris*-Form, wahrscheinlich mit weissen Blüten, gewesen ist, und dass von der Pfropfstelle eine Chimärenbildung ausgewachsen ist. Es ist ja auch möglich, dass die Form früher entstanden ist; jedenfalls dürfte die Entstehung in ähnlicher Weise stattgefunden haben.

Nach Angaben von E. OTTO (1859) und SCHÜBELER (1888) ist an *Syringa chinensis* einmal ein Ast beobachtet worden, der mehrere Blütenrispen trug, die augenscheinlich völlig mit *S. persica* übereinstimmten. In einem anderen Fall ist ein »Sport« von einer anderen *chinensis*-Sorte, var. *Saugeana*, aufgetreten (HENRY, 1901). Ist denn auch *S. chinensis* eine Chimäre? Wegen der grossen Seltenheit solcher Veränderungen ist dies nicht anzunehmen. Übrigens ist hervorzuheben, dass nach späteren Untersuchungen (TISCHLER, 1930) die als *S. persica* angebaute Form tatsächlich gleichwie *S. chinensis* eine Hybride zwischen

der Wildform von *S. persica* und *S. vulgaris* darstellt; die erwähnten Abänderungen stellen also nur Übergänge von einer Form zu einer anderen innerhalb derselben Hybridenserie dar; wie sie erklärt werden sollen, ist wohl den Zytologen zur Entscheidung zu unterbreiten.

Bei einer Farbenvarietät von *Syringa chinensis*, var. *metensis*, scheinen jedoch nach den Angaben der Literatur Rückschlüsse zur typischen Blütenfarbe ziemlich häufig vorzukommen (MC KELVEY, 1928, S. 421; nach L. HENRY). Vermutlich handelt es sich hier um eine Chimäre; selbst habe ich diese Form nicht untersucht. Ihre Entstehung ist genau bekannt; sie entstand als ein »Sport« auf einem Exemplar von *S. chinensis* in Metz um das Jahr 1860 (MC KELVEY, a. a. O.). Wenn diese Form — wie zu vermuten ist — eine Periklinalchimäre darstellt, dann ist sie also eine Mutationschimäre, in der die beiden Komponenten sich nur in der Blütenfarbe von einander unterscheiden; sie ist also von einem anderen und gewöhnlicheren Typus als *S. correlata*, die zu der seltenen Gruppe der echten »Pfropfbastarde« gehört.

### SUMMARY.

*A periclinal chimaera in the genus Syringa.* — An old garden-form of *Syringa*, generally called *S. chinensis* var. *alba*, is often reported to give inflorescences, flowers or flower-sections of the typical *S. chinensis* (BRAUN, SCHÜBELER, HENRY), and the author has also seen such reversion in the Botanical Garden of Lund. In one case only half a flower had the colour of *S. chinensis* (Fig. 1 c). The form has leaves that are of quite the same shape as in *S. chinensis*, whereas the flowers (Fig. 1 a—b) are more reminiscent of *S. vulgaris* (Fig. 1 f—g) in the cucullate flower-lobes and the round incisions of the calyx. Its flowers are of a faint lilac colour, especially pale in the lobes. The reversion (Fig. 1 d—e) are typical *S. chinensis* in shape as well as in colour. This makes it probable that the plant is a chimaera of this species and a *vulgaris*-like form. Confirmation of this is afforded by morphological and anatomical facts. The epidermis of the var. *alba* agrees with *S. vulgaris*. In the calyx and the pedicels it is green and glandular hairy as in this species, while *S. chinensis* has a reddish calyx with glandular dots there as well as on the pedicels. The lower epidermis of the leaves (Fig. 2 a—c) has — seen from the surface — curved walls in var. *alba* as in *vulgaris*, in *S. chinensis* almost quite straight ones. Under the epidermis the tissues of var. *alba* agree with *S. chinensis*; the differences

between these two forms and *S. vulgaris* are, however, not great. In *S. chinensis* and var. *alba* the proportion between the two palisade layers in the leaves is generally different from that in *S. vulgaris* (Fig. 2 *d—f*), and the collenchyma of the bark has generally more regular layers and thicker periclinal walls in relation to the anticlinal ones (Fig. 2 *g—i*). These facts, together with the meristematic conditions of *Syringa*, elucidated by SCHMIDT, speak in favour of the var. *alba* being a haplochlamydous chimaera. The inner component is *Syringa chinensis*, the outer probably a white *S. vulgaris*. Under these circumstances it should have a name of its own, most correctly the old name of A. BRAUN, *S. correlata*. It has probably arisen through grafting of *S. vulgaris* on *S. chinensis*.

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# COMBINATION EXPERIMENTS WITH MUTANT STRAINS OF OPHIOSTOMA MULTIANNULATUM

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THE fungus that was the object of the following study was isolated in 1924 by HEDGCOCK from pine lumber, and described in 1935 by DAVIDSON and HEDGCOCK (DAVIDSON, 1935) under the name of *Ceratomyces multiannulatus*, a name, however, which according to NANNFELDT (MELIN and NANNFELDT, 1934) should be exchanged for *Ophiostoma multiannulatum*. The species in question is obviously attached to the earlier known *O. pluriannulatum* (HEDGC.) H. and P. SYD., and like this (GREGOR, 1932) it is heterothallic (ANDRUS, 1936). As is shown by ROBBINS and MA (1942) as well as by FRIES (1943), *O. multiannulatum* is capable of assimilating a synthetic nutrient solution if — besides sugar and the usual inorganic salts — it contains vitamins thiamin (vitamin B<sub>1</sub>) and pyridoxin (vitamin B<sub>6</sub>). By x-ray irradiation, however, it is possible to produce mutations requiring a further addition of some vitamin, a purin, a pyrimidine, etc., to the substratum (FRIES, 1945, 1946 b).

The interesting observations of BEADLE and COONRADT (1944) as to the formation of heterocaryotic mycelia in *Neurospora crassa* led us to investigate whether heterocaryosis of the same type could appear in *Ophiostoma* too. As components of the combination experiments some mutants (or descendants of mutants) were chosen, characterized by their inability to synthesize uracil (Nos. 460, 1178 and 1208), hypoxanthine (Nos. 513, 636, 870), adenine (Nos. 1174 and 1202), or guanine (No. 848), all of them obviously without a tendency towards adaptation.

The isolation of hyphal tips was performed in the following way. Dry sterilized cover-glasses were immersed into a hot, sterile agar-solution consisting of 1,5 % washed agar, 0,2 % glucose, 0,5 % NH<sub>4</sub>-tartrate, 0,1 % KH<sub>2</sub>PO<sub>4</sub>, 0,05 % MgSO<sub>4</sub> · 7H<sub>2</sub>O, 0,01 % NaCl, 0,01 % CaCl<sub>2</sub>, 40 γ/lit. of vitamin B<sub>1</sub>, 40 γ/lit. of vitamin B<sub>6</sub>, and traces of Fe, Zn and Mn. Immediately after that, every cover-glass was put into a sterile petri dish, where the nutrient solution solidified into a thin film on the

surface of the cover-glass. A piece of the mycelium which was to be investigated was inoculated on this film. By using a thin agar film and a percentage of glucose ten times lower than that otherwise used, the mycelium growing out on the cover-glass became so loose that, by the aid of a platinum needle, individual tips of hyphae could be isolated directly under the microscope.

In isolating a hyphal tip a square piece was cut out of the agar coat in which the tip was growing, and this piece was transferred to a petri dish containing transparent malt-agar (impurities were removed by centrifugation). The growth of the hypha on the agar plate was followed by a microscopic examination every sixth hour, by which it could be controlled that no other tip of hypha, or any conidium, had through an oversight been carried along in the isolation. When the tip had ramified and grown out to a suitable length in the surrounding malt-agar, it was isolated once more with a platinum needle in the same way as before. The piece with the hyphal tip thus isolated twice was finally transferred to a culture tube containing malt-agar.

The isolation of conidia was carried out in the usual manner (FRIES, 1946 a, p. 128).

In the first experiment two (—)-mycelia, 513:14 (hypoxanthine-heterotrophic) and 848:20 (guanine-het.), were combined on malt-agar. A mixed mycelium was obtained, and when an inoculum of this was transferred to a synthetic nutrient solution, it grew there quite well. Eight cultures obtained from isolated unramified hyphal tips behaved in the same manner. Since it could be the question of heterocaryotic mycelia, also ten conidia were isolated and allowed to grow out into mycelia. These too, however, turned out to react as quite normal mycelia of the «wild type». Either the conidia were heterocaryotic too, or a reduction division had occurred resulting in a segregation of physiologically normal ascospores — prototrophic, according to the terminology of RYAN and LEDERBERG (1946). The former alternative being true, the conidia must contain several nuclei, which was not very probable, the latter alternative presupposed an «illegitimate» copulation since the components of the mycelium both represented the same incompatibility group.

In combining two (+)-mycelia, 848:5 (guanine-heterotrophic) and 460 (uracil-het.), a result was won which wholly agreed with that just described.

If the conidia and the mycelia developed from these (in the experiment first reported) were heterocaryotic, some of the ascospores

produced by mating such a mycelium with a prototrophic strain must give rise to hypoxanthine-heterotrophic mycelia, some other ascospores must produce guanine-heterotrophic mycelia, and the remaining spores, prototrophic mycelia. One of the above-mentioned 1-conidium-mycelia, obtained from the combination 513:14 + 848:20, produced perithecia with the (prototrophic) test-mycelium No. 1 (+). Seventy-nine 1-ascosporous mycelia were isolated, and their demand for purins was tested. It appeared that all were prototrophic. As ascospores have one nucleus each, heterocaryosis is excluded. Consequently both parental mycelia — thus the 1-conidium-mycelium too — must have been prototrophic and not heterocaryotic.

An illegitimate copulation must accordingly have occurred between the two mutant (—)-mycelia in the combination culture mentioned above. To get an idea of the frequency of such illegitimate copulations, 37 combinations were made between different uracil-, hypoxanthine-, adenine- and guanine-heterotrophic mutants belonging to the same incompatibility group. In some cultures, formations were observed which could be presumed to be rudiments of fruit-bodies. Completely developed fruit-bodies did not appear in any single case, and have in fact never been observed in our laboratory in monosporic cultures, or in cultures of the (+) × (+)- or (—) × (—)-type. In this respect *O. multiannulatum* wholly corresponds to *Neurospora*.

When the combination cultures in question were 50 days old, two big pieces of the mycelial mat together with the agar layer immediately beneath were removed, and one was transferred to a flask containing a sterile, synthetic nutrient solution (without any addition of nucleotide components), the other was fixed in pikroformol, according to BOUIN-MAIRE. In 23 of the flasks inoculated in this manner the inoculum remained rather unaltered; only by degrees did solitary hyphae slowly develop; in 14 flasks, on the other hand, an intensive development of mycelium immediately set in, so that the whole volume of the solution was interwoven with hyphae even in a few days (Table 1). In the latter cases the combination cultures had given rise to prototrophic mycelia. In some of these cases it was controlled that it was not the question of heterocaryotic mycelia.

The fixed pieces of mycelium were microtomed, stained with iron haematoxyline and lichtgrün, and examined as to the occurrence of rudiments of perithecia. Such rudiments turned out to be present in all the cultures giving rise to prototrophic mycelia. Thus, there hardly seems to be any doubt about the prototrophic mycelia having arisen

TABLE 1. *The segregation of prototrophic mycelium in illegitimate combinations between different mutant strains of Ophiostoma multi-annulatum, and the growth rate of the combinations on agar with minimum nutrient solution.*

a = adenine-heterotrophic; g = guanine-heterotrophic; h = hypoxanthine-heterotrophic; u = uracil-heterotrophic.

In the second column + means that prototrophic mycelium was developed, — means that no development of prototrophic mycelium was observed. In the last column . means that no determination of the growth rate was made.

Strains	Development of prototrophic mycelium	Growth rate of the combination in mms. per day
513 (+, h) + 848: 5 (+, g) . . . . .	—	0
513 (+, h) + 848: 15 (+, g) . . . . .	+	0
636 (+, h) + 848: 5 (+, g) . . . . .	—	0, <sub>8</sub>
870 (+, h) + 848: 5 (+, g) . . . . .	—	0
870 (+, h) + 848: 15 (+, g) . . . . .	+	0
513: 1 (—, h) + 848 (—, g) . . . . .	—	0
513: 14 (—, h) + 848 (—, g) . . . . .	—	0
513: 1 (—, h) + 848: 20 (—, g) . . . . .	—	0
513: 14 (—, h) + 848: 20 (—, g) . . . . .	+	2, <sub>1</sub>
1174 (+, a) + 848: 5 (+, g) . . . . .	—	.
1174 (+, a) + 848: 15 (+, g) . . . . .	—	.
1202 (+, a) + 848: 5 (+, g) . . . . .	—	0
1202 (+, a) + 848: 15 (+, g) . . . . .	—	0
513 (+, h) + 460 (+, u) . . . . .	—	0, <sub>9</sub>
513 (+, h) + 460: 9 (+, u) . . . . .	+	0
513 (+, h) + 1178 (+, u) . . . . .	+	0
513 (+, h) + 1208 (+, u) . . . . .	—	0
870 (+, h) + 460: 9 (+, u) . . . . .	+	0, <sub>5</sub>
870 (+, h) + 1178 (+, u) . . . . .	+	0
870 (+, h) + 1208 (+, u) . . . . .	—	0, <sub>4</sub>
513: 1 (—, h) + 460: 4 (—, u) . . . . .	—	0
513: 14 (—, h) + 460: 4 (—, u) . . . . .	—	0
1174 (+, a) + 460: 9 (+, u) . . . . .	—	.
1174 (+, a) + 1178 (+, u) . . . . .	—	.
1174 (+, a) + 1208 (+, u) . . . . .	—	.
1202 (+, a) + 460: 9 (+, u) . . . . .	—	0

Strains	Development of prototrophic mycelium	Growth rate of the combination in mms. per day
1202 (+, a) + 1178 (+, u) . . . . .	—	0
1202 (+, a) + 1208 (+, u) . . . . .	—	0
848: 5 (+, g) + 460 (+, u) . . . . .	+	.
848: 5 (+, g) + 460: 9 (+, u) . . . . .	+	0,7
848: 5 (+, g) + 1178 (+, u) . . . . .	—	0,7
848: 5 (+, g) + 1208 (+, u) . . . . .	+	0,3
848: 15 (+, g) + 460: 9 (+, u) . . . . .	+	0,5
848: 15 (+, g) + 1178 (+, u) . . . . .	+	0,5
848: 15 (+, g) + 1208 (+, u) . . . . .	+	0,2
848 (—, g) + 460: 4 (—, u) . . . . .	—	0,6
848: 20 (—, g) + 460: 4 (—, u) . . . . .	+	1,0
1 (+, prototrophic) . . . . .	(+)	4,1
2 (—, prototrophic) . . . . .	(+)	4,7

from ascospores formed after reduction division. Such illegitimate copulations and reduction divisions are, however, obviously rare. No clear reduction division could be observed in any of the slides, nor did a segregation of normal mycelia take place in all the cultures where rudiments of perithecia appeared.

Thus, the existence of illegitimate copulations in *O. multiannulatum* would undoubtedly have been very difficult to establish, unless the copulants combined here had been of such a sort that a certain product of segregation — the prototrophic mycelium — could easily be identified by its physiological properties.

In the experiments mentioned the illegitimate copulations rendered it however impossible to decide whether heterocaryosis occurs in *O. multiannulatum*. To prevent these copulations, if possible, experiments were made to combine different types of mutants on a synthetic agar medium of the composition stated on a foregoing page, but with 2 % glucose instead of 0,2 %. The growth of the mutants on this substratum was extremely poor and was caused by occurrence of inconsiderable traces of the respective nucleotide constituents in the inocula and in the substratum. Rudiments of fruit-bodies did not occur in any case. In the 31 combination cultures arranged with different mutants (Table 1) a rather vigorous formation of mycelium set in after

two weeks, at the earliest, in 13 of the cultures. The growth rate was, however, very low, and considerably inferior to the prototrophic mycelia on this substratum.

One of these combination cultures, 636 (+, hypoxanthine-heterotrophic)  $\times$  848:5 (+, guanine-het.), was more closely examined. All of 9 isolated hyphal tips as well as all the conidia developed into hypoxanthine-heterotrophic mycelia. Consequently, no reduction division had taken place in this case, nor had a heterocaryotic mycelium arisen; but the growth was probably conditioned by an extracellular interchange of hypoxanthine and guanine in the same way as has earlier been described in so-called artificial symbioses (KÖGL and FRIES, 1937; SCHOPFER, 1938; »extracellular symbiosis», according to BEADLE and COONRADT, 1944). That no guanine-heterotrophic conidia and tips of hyphae, but merely hypoxanthine-heterotrophic ones were obtained, probably depends on the fact that the hypoxanthine-heterotrophic component is the more vital and rapidly growing one in the combination. Presumably the guanine-heterotrophic partner limits the growth of the system.

In the combination between a hypoxanthine- and a uracil-heterotrophic mutant, viz. 513 (+) and 460 (+), all the four hyphal tips and nine conidia isolated were also hypoxanthine-heterotrophic. This component, then, is the most rapidly growing one even in this combination.

The possibility can of course not be excluded that also heterocaryotic hyphae are present in these combination cultures. It is obvious, however, that heterocaryotic mycelia having a growth rate equivalent to that of prototrophic mycelia cannot be formed in *O. multiannulatum* — unlike *Neurospora* — at least not under the conditions tested here. This may perhaps be due to the fact that the species of *Ophiostoma* — to judge from available particulars and figures in literature (MITTMANN, 1932; ANDRUS, 1936) — possess uninucleate cells, whereas *Neurospora*, like perhaps most other Ascomycetes, is a coenocyte with numerous nuclei in each cell.

The capacity of forming heterocaryotic mycelia characteristic of *Neurospora* has, as is known, proved to imply a very convenient means of deciding whether two gene mutations concern one and the same gene. This possibility seems to be excluded in *O. multiannulatum*. So in the first place one is here restricted to using the ordinary method with matings between mutants (or descendants of mutants) belonging to different incompatibility groups and to an examination of the properties of the progeny — fairly easily done, it is true, when it mainly

comes to indicating the absence or presence of physiologically normal, prototrophic products of segregation. From a certain mutant mycelium it may, however, often be difficult and time-consuming to produce — by crossings with normal test-mycelia — descendants of the opposite mating-type necessary for such combination experiments. In so far as illegitimate copulations between mutants within the same incompatibility group prove the existence of non-allelism by a segregation of prototrophic mycelium this roundabout way in solving such problems can be avoided.

*Summary.* — In 14 of 37 illegitimate combinations of different physiological (uracil-, hypoxanthine-, adenine- and guanine-heterotrophic) mutants of *Ophiostoma multiannulatum* on malt-agar a prototrophic (= »wild type») mycelium developed. It is supposed that this must have arisen through a segregation in rudiments of perithecia. Heterocaryotic hyphae could not be found in these combinations, nor in such combination cultures on agar containing a synthetic nutrient solution. In 13 of these 31 cases last mentioned, however, there was some growth probably due to the formation of extracellular symbioses.

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# CONSTITUTION AND C-MITOTIC ACTIVITY OF ISO-COLCHICINE

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## I. INTRODUCTION.

COLCHICINE was isolated from *Colchicum autumnale* for the first time by ZEISEL. Through the work of this author, continued during a long period (ZEISEL, 1883, 1886; JOHANNY and ZEISEL, 1888; ZEISEL and v. STOCKERT, 1913), the empirical formula of colchicine was determined as  $C_{22}H_{25}O_6N$ . The function of the nitrogen atom as well as of five of the oxygen atoms was understood. The work of WINDAUS (e. g., 1924) and collaborators led to the constitution of formula (I) (see Scheme 1), which formula, however, does not account for all the properties of colchicine.

That colchicine has a remarkable action on mitosis was early known. Its cytological effect was studied in animal material by DUSTIN (e. g., 1934), in plant material by GAVAUDAN and GAVAUDAN (1937) and by DUSTIN, HAVAS and LITS (1937), and later on by a great many workers. The main effect of colchicine on mitosis is a more or less complete destruction of the spindle apparatus. The chromosomes divide as normally, but their daughter halves are not distributed to the poles. This deviation from the normal course of mitosis gives a very characteristic morphological picture, which in its main features is identical in all animals and plants studied. It was called c-mitosis by LEVAN (1938), which rather neutral term seems preferable to the more or less descriptive or hypothesizing terms, e. g., caryoclastic mitosis, stathmokinesis, polyploidogenic action, mitosis inhibition, mitosis poisoning, each of which may cover only certain sides of the reaction.

During later years a whole literature has grown up around the colchicine problem, especially since the polyploidogenic action of colchicine was detected by BLAKESLEE (1937). Most of these papers deal with cyto-genetic aspects, as the appearance and behaviour of the new artificially produced polyploids. The morphology of the cytological effect on the living cell has also received due attention, most workers being in agreement as to the main features of the c-mitosis.

Among the lines of research the outcome of which cannot yet be regarded as settled, reference may be made to the important question as to what part of the colchicine molecule is responsible for its activity (BRUES and COHEN, 1936; COOK and ENGEL, 1940; LETTRÉ, ALBRECHT and FERNHOLZ, 1941; LETTRÉ and FERNHOLZ, 1943). Other recent investigations have centred around the striking lack of specificity of the c-mitotic reactions. It has been found that many substances of quite different chemical character from colchicine nevertheless show c-mitotic activity. Thus, acenaphthene was found to be active (KOSTOFF, 1938) and likewise apiol (GAVAUDAN and GAVAUDAN, 1939). LEVAN and ÖSTERGREN (1943) and ÖSTERGREN and LEVAN (1943) tested series of related substances, and by means of a quantitative method it was possible to decide that as a rule some concentrations of most substances gave the reaction. This was true of all halogen and methyl derivatives tested of benzene, naphthalene, cyclohexane and thiophene. Activity was present especially in substances known for their narcotic properties (ÖSTERGREN, 1944). The apparent contradiction between, on one hand, specifically acting chemical groups and, on the other hand, the striking lack of specificity of the c-mitotic reactions may be overbridged, as will be discussed further in a later chapter.

The present writers intend to deal in a few papers with some problems connected with the c-mitosis, problems often lying on the borderline between biochemistry and cytology. This first communication will give some data concerning the action on *Allium* roots of an isomeric form of colchicine. The method employed is the same as earlier adopted at this laboratory, i. e. a determination of the effect of different concentrations of the substance on growing root-tips of *Allium Cepa* bulbs. Fixation of the root tips has been made at different intervals after the beginning of the treatment.

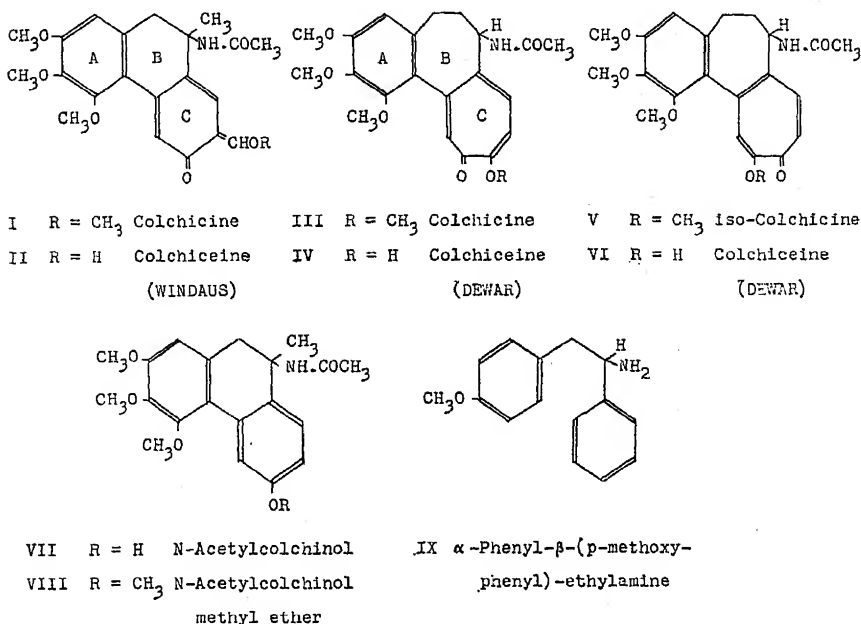
Our thanks are due to Dr. M. SORKIN, Basle, for presenting us with a sample of *iso*-colchicine. The stay of E. STEINEGGER at Svalöf has been made possible by a fellowship from the Swiss »Stiftung für biologisch-medizinische Stipendien».

## II. THE CHEMICAL CONSTITUTION OF COLCHICINE AND ISO-COLCHICINE.

It has been shown that the constitution of colchicine as given by WINDAUS is unsatisfactory. BARTON, COOK and LOUDON (1945) claim that the ring *B* of colchicine must be seven-membered. DEWAR (1945 a,

b, c) supposes that the ring C is also seven-membered, just as is the case with the stipitatic acid isolated from *Penicillium stipitatum*. Through combination of the two seven-rings B and C DEWAR (1945 b) proposed the constitution (III) as being the most probable one for colchicine.

The methyl group of ring C is easily split off, colchiceine thereby originating constitution (II) according to WINDAUS, (IV) or (VI) according to DEWAR. While the constitution (II) of WINDAUS leaves open only one possibility for the formula of colchiceine (the possibility of cis-trans-



Scheme 1. The constitution of colchicine and related substances.

isomery is not taken into account), the constitution given by DEWAR allows of two resonating structures. This is perfectly analogous to the two resonating constitutions known for stipitatic acid (DEWAR, 1945 a).

If colchiceine is methylated, two isomeric methylated compounds would be expected, as indeed they have been obtained from stipitatic acid. Their structures would correspond to formulae (III) and (V). MEYER and REICHSTEIN (1944), after methylating colchiceine with diazomethane, obtained crystalline colchicine to a yield of two per cent with an optical rotation of  $[\alpha]_D^{19} = -122.4^\circ \pm 2^\circ$ , while the bulk of the reaction product remained amorphous and showed an optical rotation of

$[\alpha]_D^{24} = -223.5^\circ \pm 2^\circ$ . The latter substance showed the same analytical composition as colchicine.

Recently SORKIN (1946) succeeded in separating two substances from the mixture originating after the reaction of diazomethane on colchicine. Besides normal colchicine one isomere of colchicine, called *iso-colchicine*, was also obtained in crystalline form for the first time. *Iso-colchicine* is described by SORKIN as a colourless powder, crystallizing into orthogonal or hexagonal plates. Melting point:  $225-226^\circ$ , optical rotation:  $[\alpha]_D^{12} = -306.7^\circ \pm 3^\circ$ . In similarity to colchicine it dissolves in aqueous mineral acids with yellow colour. On heating together with mineral acids it again gives colchicine.

### III. THE C-MITOTIC ACTION OF ISO-COLCHICINE.

The action on *Allium* roots of a dilution series of *iso-colchicine*, including 7000, 1000, 100 and  $10 \times 10^{-6}$  mol/l, immediately revealed one striking difference from colchicine. In normal colchicine concentrations of 100 to  $150 \times 10^{-6}$  mol/l already give c-mitosis, in *iso-colchicine* none of the above concentrations gave complete c-mitosis. In the strongest,  $7000 \times 10^{-6}$  mol/l, there were tendencies to c-mitosis. Fig. 1 *b-d* represents three stages of increasing c-mitotic tendencies after four hours' treatment with  $1000 \times 10^{-6}$  mol/l (*b*) and with  $7000 \times 10^{-6}$  mol/l (*c-d*). Compared with the normal mitosis of Fig. 1 *a*, taken from a 24 hours' treatment with  $10 \times 10^{-6}$  mol/l, it will be noticed that the chromosome contraction is more pronounced in  $7000 \times 10^{-6}$  mol. Even the spindle begins to show irregularities. In *b* it is rather regular, in *c* the poles are flattened out, showing traces of multipolarity. The chromosomes are still gathered in an equatorial plate. In *c* this plate is quite irregular. Since the plasm in this cell showed a heavy staining, the spindle fibres were well visible. Several individual poles were present, and those small bundles of spindle fibres which are in contact with the centromeric regions of the chromosomes were oriented in different directions. Complete destruction of the spindle did not occur in this concentration.

On fixing after 24 hours' treatment it turned out that all concentrations, even the strongest one, showed exclusively normal mitoses. The c-mitotic effect shown by  $7000 \times 10^{-6}$  mol/l shortly after the beginning of the treatment thus depended on a shock action, early disappearing. No poison effect was noticed in these concentrations. The

roots were still turgescient after seven days, even if no further growth had occurred.

After the above results were known two higher concentrations were

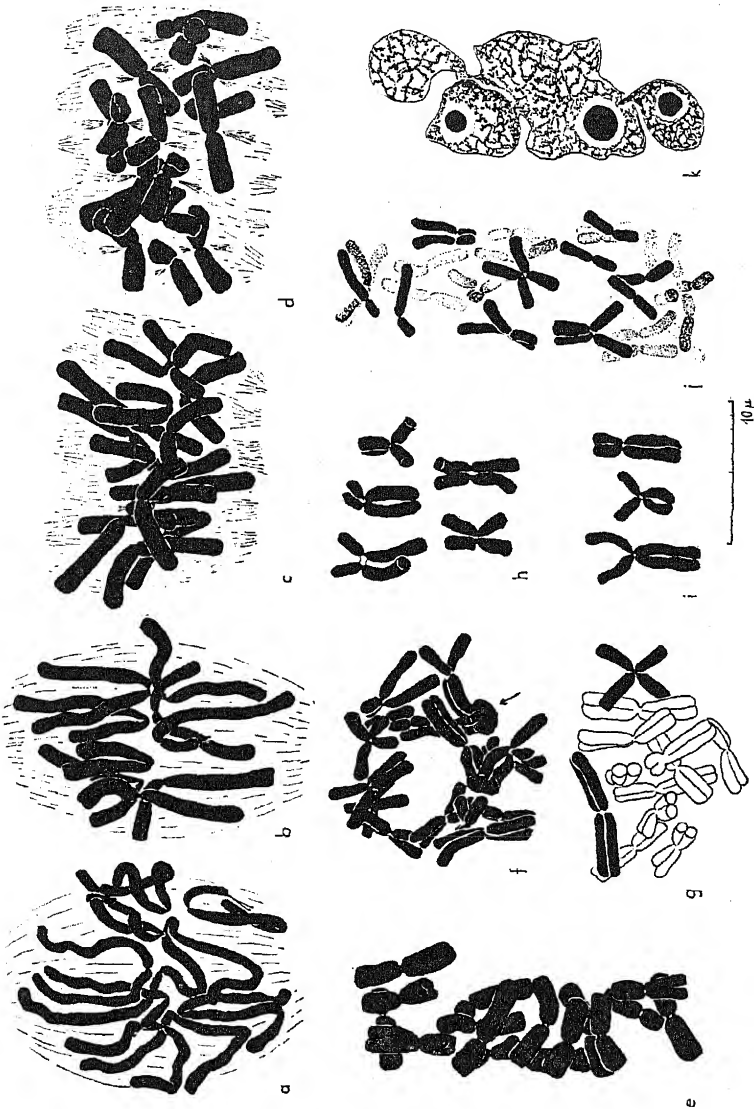


Fig. 1. *Allium Cepa* mitoses in the root-tips. — a: normal metaphase; b—d: increasing c-mitotic effect on the chromosomes; e—g: full c-metaphase; h—i: c-pairs from two cells, separately drawn; j: c-anaphase; k: lobated resting nucleus resulting from c-mitosis; a: 10, b: 1000, c—d: 7000, e—k:  $14000 \times 10^{-6}$  mol iso-colchicine; b—e, g, k: 4 hours; a, f, h—j: 24 hours' treatment. —  $\times 2000$ .

tested, viz.  $25000$  and  $14000 \times 10^{-6}$  mol/l, i. e. 1 and 0,56 %. The higher of these, which corresponds to a semi-saturated solution, gave a distinct poison effect after 4 hours, and only few mitoses were present. These

were to a large extent c-mitoses. A decided stickiness was seen, the mitoses belonging to the compact, agglutinated type. After 24 hours no divisions were found in this concentration, after two days a few mitoses were again found, peculiarly enough they now had normal spindles and did not show especially strong contraction. After four days most of the meristem did not take the stain and the nuclei had no visible structures. The cells were probably not dead, however, the roots still remaining turgescient after 11 days. After 22 days the roots began softening, and no growth occurred.

The cytological study of the concentration  $14000 \times 10^{-6}$  was started after two hours' treatment. The mitoses were then normal but showed clear c-mitotic tendencies. After four hours most of the mitoses had turned into typical c-mitoses. Fig. 1 *e* is an instance of such a mitosis, the chromosomes of which are strongly contracted; the chromosomes show no equatorial arrangement and no traces of any spindle can be detected. The centromeres are probably still undivided, although each centromere is parted into two darker grains lying in line with the two chromatids. Between these stained parts the centromeres are lighter. It may be that the centromeres are already divided, the chromosomes, however, being held together by their stickiness.

A period of 24 hours in  $14000 \times 10^{-6}$  mol/l induces complete c-mitosis; normal spindles are totally absent in the slide. Different stages of the c-mitosis resulting from this treatment are pictured in Fig. 1 *f* and *h—j*. The c-mitosis here must be judged as complete and typical. It is characterized, however, by a higher degree of stickiness than is present in c-mitosis of, for instance, normal colchicine. This stickiness brings about an agglutination of the c-pairs of the c-metaphase. Sometimes two or more c-pairs stick together in one end at a drop of chromatin, which seems to have developed from the chromosome arms involved (see Fig. 1 *f*, at the arrow). A certain irregularity perceived in the development of the c-pairs is probably due to this stickiness. Thus, in the same cell there often occurred c-pairs with their chromatids quite parallel, sticking together along their whole length, besides typical x-shaped c-pairs. Sometimes the daughter chromatids of one c-pair may stick together in one half and be separated in the other half (Fig. 1 *h*, *i*). Such irregularities were still present after four days, as shown by Fig. 1 *g*. This shows one cell with one x-shaped c-pair, while all the other c-pairs have their chromatids sticking together parallelly. This difference in the appearance of the c-pairs may be of the same nature as that found by WITKUS and BERGER (1944). In their experiments

colchicine gave the ordinary x-shaped c-pairs, while veratrine gave c-pairs of the more sticky type.

In Fig. 1 j a c-anaphase is represented. At this stage, too, the course is somewhat more irregular than in ordinary c-mitosis. Most c-pairs of the picture are already separated into »ski pairs», but one or a couple are of x-shape and evidently hang together at their centromeres. Probably as a result of the stickiness, the division of the centromeres does not cause a simultaneous partition of the chromosomes. Or else the heavy treatment has upset the synchronization of the division of the centromeres. In normal c-mitoses the c-anaphase always starts absolutely at the same time in all c-pairs, while in c-mitosis induced by *iso*-colchicine the timing of the process seems to be less exact.

Fig. 1 k shows one lobated nucleus formed from a c-telophase in which the c-pairs must have been widely scattered. This type as well as multinuclear cells commonly occurred after a treatment of  $14000 \times 10^{-6}$  mol/l for four days. A slight poison effect was met with in this treatment, but the roots maintained their turgescence all the time. Most mitoses were of c-type at this time. The macroscopical colchicine reaction, the swelling-up of the growth region of the roots, the so-called c-tumour reaction, was carefully looked for all through our experiments. The reaction was found only in one concentration, viz.  $14000 \times 10^{-6}$  mol/l, in which it began to appear after three days. Fig. 2 shows the appearance of the c-tumours after five days. Undoubted c-tumours are seen clearly in the

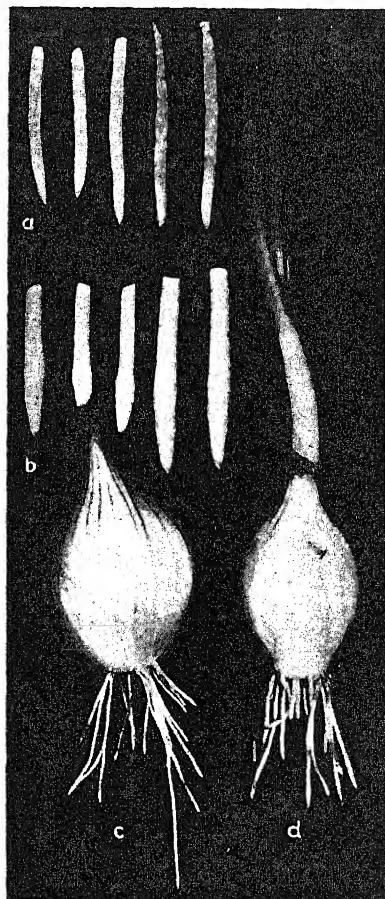


Fig. 2. *Allium Cepa*. — a, c: untreated, b, d: treated with  $14000 \times 10^{-6}$  mol *iso*-colchicine; a, b: enlarged root-tips from c and d respectively.

enlarged roots of Fig. 2 *b*, especially when compared with the untreated roots of Fig. 2 *a*. The reaction is, however, less pronounced than after treatment with ordinary colchicine; the tumours are smaller and their shape is more elongated. It must be considered of some interest that the two reactions, c-mitosis and c-tumour, accompany each other in *iso*-colchicine just as in colchicine, although in the case of *iso*-colchicine in 100 times higher concentrations.

Here follows a survey of the observed occurrence of c-mitosis in *iso*-colchicine (+ = c-mitosis, ± = mixed c- and normal mitosis, — = normal mitosis, 0 = not studied):

Concentration in 10 <sup>-6</sup> mol/l	2 hours	4 hours	24 hours	2 days	4 days
25000 . . . . .	0	(+)	?	(—)	?
14000 . . . . .	(+)	±	+	0	(+)
7000 . . . . .	0	(+)	—	0	0
1000 . . . . .	0	—	—	0	0

#### IV. DISCUSSION.

Although certain small morphological differences seem to be present between the c-mitosis of colchicine and that of *iso*-colchicine, the same main type undoubtedly prevails in both substances. On the other hand, the differences in threshold values are striking: in *Allium* colchicine gives c-tumours and c-mitoses even in concentrations a little above  $100 \times 10^{-6}$  mol/l, *iso*-colchicine gives tendencies to c-mitosis in 7000 and full effect in  $14000 \times 10^{-6}$  mol/l. The threshold is heightened 70 to 100 times in the *iso*-form.

LEVAN and ÖSTERGREN (l. c.), studying the c-mitosis of several hydrocarbons, found that, especially within related series, the activity thresholds are negatively correlated to the water solubility of the substances. The conclusion was drawn that the primary action on the cell was exerted physically and not chemically. It was stressed, however, that colchicine occupied an exceptional position among the c-mitotic substances owing to its high activity and high water solubility. GAVAUDAN, DODÉ and POUSSEL (1944), working partly on the material of LEVAN and ÖSTERGREN and partly on their own material, sharpened the opposition between, on one hand, colchicine and a few other readily soluble substances, and, on the other hand, the hydrocarbons and substances of low solubility. They put the c-mitotic action in relation to the thermodynamic activity of the c-mitotic substances.

This activity may be calculated with sufficient accuracy as the relation between the active concentration and the concentration of saturated water solution. To FERGUSON (1939) falls the merit of having introduced this entity in biology. By calculating the thermodynamic activity of certain narcotics, poisons, insecticides and bactericides he found that the substances generally fell into two well-defined groups: (1) The activity is directly correlated to physical properties of the substances, such as solubility or vapour pressure. In this group the threshold seldom lies below 0,1 of the water solubility, and since it cannot exceed 1 the thermodynamic activity varies very little, although the absolute values of the active concentrations may vary several thousand times. FERGUSON considered the effect of this type of substances to be of an unspecific, physical nature. (2) Great differences occur between activity threshold and water solubility. The thermodynamic activity in this group consequently varies a great deal; in highly soluble substances it is low, often of the size order of 0,001 or less. Here FERGUSON assumed the biological activity to be due to a more specific, chemical action. FERGUSON pointed out the possibility and gave instances of substances which take an intermediate position, acting at the same time physically and chemically.

If we apply FERGUSON's ideas upon colchicine and *iso*-colchicine some interesting features come to light. We have the following values (concerning the solubility of colchicine we must refer to a later paper):

	Solubility in 10 <sup>-6</sup> mol/l	Activity threshold in 10 <sup>-6</sup> mol/l	Thermodynamic activity
colchicine . . . . .	> 500·000	150	< 0,000·3
<i>iso</i> -colchicine . . . .	50·000	14·000	0,28

It is immediately seen that colchicine and *iso*-colchicine belong to different types of substances: colchicine with its low thermodynamic activity is a typical representative of the chemically acting substances, while *iso*-colchicine with its 900 times higher thermodynamic activity belongs to the type of unspecifically acting substances. The most surprising result of the present investigation is, in fact, that such a small change in the colchicine molecule as the change from colchicine to *iso*-colchicine will so totally change the activity. Evidently, at least in the case of *Allium*, the specific or chemical activity of colchicine has been removed: for *iso*-colchicine there remains not much more than its physical activity.

In the introduction we referred to studies dealing with the question of what part of the colchicine molecule may be responsible for its

activity. Thus, BRUES and COHEN (1936) found that colchicine (IV) or (VI) (Scheme 1) only had  $1/30$  to  $1/40$  of the activity of colchicine. The activity of N-acetyl-colchicinol (VII) was similar. Its methyl ether (VIII) had only  $1/400$  of the activity of colchicine. By testing various chemical compounds on animal material LETTRÉ and collaborators (l. c.) arrived at the conclusion that  $\alpha$ -phenyl- $\beta$ -(p-methoxy-phenyl)-ethylamine (IX) was the simplest »mitosis poison» of »colchicine type». By the construction of the new colchicine formula with seven-rings this conclusion has lost somewhat in significance. Moreover, it is now a well-known fact that much simpler substances with six-rings or simple aliphatic substances have c-mitotic activity. ÖSTERGREN (1944), for instance, enumerates about 80 organic substances, all of which exhibit c-mitotic activity more or less plainly (or in some cases latently).

What answer may be given to the question as to which group of a chemical compound is the active one? By studying the thermodynamic activity of various derivatives of a substance it may be possible to decide what groups only participate in the unspecific physical activity and what groups are necessary for giving a specific chemical effect. In our case it turned out that colchicine on being transformed into *iso*-colchicine lost its specific activity. The arrangement of keto- and methoxyl-groups as in ring C of colchicine must consequently be necessary for its specific action on *Allium* roots. To what extent the other groups of the molecule also take part in the specific activity, and how far our results in *Allium* may be valid in other materials, will be discussed in a later paper of the present series.

### SUMMARY.

The c-mitotic activity of *iso*-colchicine is tested. It is found that a concentration of  $14000 \times 10^{-6}$  mol/l induces full c-mitosis and c-tumours.  $7000 \times 10^{-6}$  mol/l only giving tendencies to c-mitosis and no c-tumours. Its activity is thus about 100 times lower than that of colchicine.

FERGUSON's division of substances according to their thermodynamic activity into two groups, viz. unspecifically or physically active and specifically or chemically active, is adapted to the present case. It is claimed that colchicine has a specific activity, which gets lost on its transformation into *iso*-colchicine, this latter acting by its physical activity.

Svalöf, January, 1947.

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# SOME OBSERVATIONS ON POLLINATION AND FRUIT SETTING IN ECUADORIAN CACAO

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DURING a short stay at the Swedish Hacienda Clementina in the neighbourhood of Babahoyo, Ecuador, I had an opportunity to make some observations and experiments concerning pollination and fruit setting in *Theobroma cacao*. Four trees growing close to the hacienda and numbered 2, 3, 4 and 5 were used for the experiments. The origin of these trees is not quite clear, but they are most probably derived from crosses between »Cacao Nacional», the widespread native cacao variety of Ecuador, and trees raised from seeds imported from Venezuela.

A total of 156 flowers were pollinated, 81 with pollen from the same tree, 75 with pollen from other trees. On account of the structure of the flowers, which normally prevents spontaneous self-fertilization, it was not necessary to emasculate the flowers. However, before pollination the virginity of the stigmata was controlled with the help of a strong lens. Only flowers newly opened were used, and these were pollinated in the morning or some time before noon.

*Results of self-pollinations.* — On trees Nos. 2, 3 and 4 all self-pollinated flowers were abscised and fell to the ground before the end of the 3rd day. The numbers of pollinated flowers were 14, 29 and 13 respectively. A few of these flowers, however, were used for fixation for embryological studies before the flowers had yet fallen. Nevertheless the pollinations clearly show that these three trees are self-incompatible. The fourth tree, No. 5, on the contrary, was found to be self-compatible. Of 13 self-pollinated flowers, which were allowed to develop up to 12 days before they were fixed, 11 showed an obvious fruit setting and an even and regular development. Twelve additional self-pollinated flowers were fixed within 3 days, before it was possible to decide whether the pollination had been successful. However, all ovaries in these flowers when observed more than 24 hours after pollination showed an obvious swelling, indicating a positive result also

in these cases. Though the degree of flowering in this tree was rather sparse, fruit setting was unusually rich (Fig. 1), all stages of young fruits being represented.

Of the self-incompatible trees especially No. 3 showed a very profuse flowering, but evidently only a small fraction of these flowers gave rise to fruits. In contrast to the self-compatible tree (No. 5) young developing fruits were quite sparse.



Fig. 1. The self-compatible tree (No. 5), showing a high degree of fruit setting.

*Results of cross pollinations.* — Part of the cross pollinated flowers were used for fixations before the seed setting could be judged. The remaining flowers gave the following result:

Cross combination	Flowers abscissed	Commencing fruit development
3 × 5 .....	11	4
5 × 3 .....	7	3
3 × 2 .....	12	4
2 × 3 .....	3	2
3 × 4 .....	11	—

According to these data, a commencing fruit development was obtained in the cross self-incompatible  $\times$  self-compatible ( $3 \times 5$ ) and reciprocally ( $5 \times 3$ ). The same result was obtained in one of the crosses self-incompatible  $\times$  self-incompatible ( $3 \times 2$  and  $2 \times 3$ ), whereas the other cross of this kind ( $3 \times 4$ ) gave a quite negative result. Thus, though quite preliminary the self and cross pollinations undertaken are sufficient to demonstrate that the cacao trees at Clementina are different as regards the conditions of fertilization and fruit development. Further information may perhaps be obtained from an embryological study of the material fixed. This work is carried out by my colleague, Dr. A. HÅKANSSON.

*The degree of spontaneous pollination.* — On the self-incompatible tree No. 3, which flowered abundantly, aphides and ants were present in large numbers. In order to study the possible rôle of the aphides for pollination the frequencies of pollen grains at the styles of lousy and non-lousy flowers were compared. The flowers were collected at 5 o'clock p. m., all open flowers being taken at random. Most of these flowers had opened in the morning of the same day, but some flowers were 2 or even 3 days old. After picking, the flowers were immediately divided into two categories, i. e. flowers with and without aphides on the flowers or flower-stalks. After staining over night in a mixture of aceto-carmin and glycerine in equal parts the styles were examined under the microscope. At first dry styles were used, but staining was found to be necessary in order to distinguish the pollen grains with certainty from the small papillae which occur at the style, especially at its basal part.

When the styles are inclosed in the aceto-carmin-glycerine under pressure of the cover slips, some pollen grains are detached from the stigmata and swim free in the medium. In order to eliminate this source of error the whole slides were carefully looked through and the number of free pollen grains counted.

The five stigmatal lobes of the style were often in close contact and pressed against each other even in flowers one day old, and in such cases it was sometimes difficult to decide whether the pollen grains were situated at the stigma or at the style below the stigma. The values given below represent the total number of pollen grains observed, whether situated at the stigma or lower down at the style. A few times pollen grains situated at the side of the style and even near the base were observed to have germinated and to have produced pollen tubes.

Whether these parts of the style are really equally conceptible as the stigma lobes remains to be studied.

The observations made may be summarized as follows:

Category	Number of styles	Number of styles with pollen	Number of pollen grains	Number of pollen grains per pollinated style	Number of pollen grains per all styles
Non-lousy flowers: . .	44	14	59	4,2	1,3
Lousy » : . .	58	37	485	13,1	8,4

Of the 44 non-lousy flowers only 14 were pollinated. Of the 58 lousy flowers, on the contrary, as many as 37 had pollen. Thus, these figures clearly demonstrate that the lousy flowers were better pollinated than the non-lousy ones. The two distributions 30 : 14 and 21 : 37 are significantly different, a  $\chi^2$ -test giving a P smaller than 0,001.

The pollen counts also demonstrate that the frequency of unpollinated flowers is very high, and that the number of pollen grains at the pollinated flowers is as a rule insufficient to give fruits with a normal number of seed. The seed number per fruit was counted in four different populations of the hacienda Clementina. One ripe fruit was taken at random from each tree, the number of trees examined in this way ranging from 43 to 75. The following result was obtained:

Population	Number of seeds per fruit										n	M
	5-10	15-20	25-30	35-40	45-50	55						
La Victoria . . . . .	2	3	1	3	8	13	7	6			43	35,15
Sta Isabel . . . . .	1	2	5	6	9	15	12	14	3	1	68	32,80
San Rafael . . . . .	1	1	3	4	9	5	12	28	17	5	85	38,60
Nacional . . . . .	2	6	7	5	10	17	18	8	2		75	35,15

Evidently the number of seeds per fruit is rather variable, the individual values ranging from 5 to 55 with average values from 32,80 to 38,60. The distributions in each series show a rather pronounced skewness, the maximum frequency class being rather close to the class representing the highest seed number.

The variation in seed number per fruit may be due to three causes, (1) a variable number of functional ovules, (2) a variation in the number of ovules which are fertilized, and (3) a variation in the number of fertilized ovules which develop normally. The pronounced skewness of the curves strongly indicates that the second cause is the most important factor. The number of functional ovules is probably close to the maximal number of seeds, but if pollination is insufficient a variable number of these ovules will not be fertilized.

According to the values given above, 51 flowers (14 + 37) had been pollinated, the average number of pollen grains per pollinated style being 4.2 in the non-lousy flowers and 13.1 in the lousy ones. It is of interest to analyse the distribution of the pollen grains on the different styles. The following values were obtained:

Slide No.	Number of pollen grains at the stigma or style									Number of free grains
	1 —	5 —	10 —	15 —	20 —	25 —	30 ...	70 —	80	
1 (lousy) . . . . .	5	2	—	1						4
2 » . . . . .	8	1	—	1	1					72
3 » . . . . .	3	—	3	—	—	—	—	1		36
4 » . . . . .	7	1	1	1	—	1				76
5 (non-lousy) ...	2	—	1							33
6 » ...	4	—	1							0
7 » ...	2									0
8 » ...	3	1								3
Total	34	5	6	3	1	1	—	1		224

Of the 51 styles observed to be pollinated only one had with certainty received a surplus of pollen (more than 70 grains). A few of the other ones had also received a sufficient number of pollen grains for fruit development but probably not for the maximal or even the average number of seeds.

Though the presence of free pollen grains, being detached from the styles, is a disturbing source of error the counts nevertheless demonstrate that the majority of the pollinated flowers had received less than 5 pollen grains. This figure also includes grains not situated at the stigma but at other parts of the style. Such a low number of pollen grains may perhaps be able to start fruit development, but only in quite rare cases will this result in a ripe fruit.

In cacao it is a regular phenomenon that a considerable proportion of the young fruits stop development a few weeks after pollination and die when they have reached a length of a few cm. This cherelle wilting seems to be a normal physiological phenomenon caused by the limited ability of the tree to bear fruit.

HUMPHRIES (1941) has shown that cherelle wilt is primarily due to competition between pods for water and nutrients. Considering the present observations, which show that most of the pollinated styles receive a quite insufficient number of pollen grains, it is tempting to assume that cherelle wilting is largely caused by incomplete pollination.

POUND (1931 a), however, tested this possibility and did not find any difference in the number of developing ovules in wilting and normal cherelles having a length of two inches. On the other hand, POUND also observed that in ovaries examined about 6 days after pollination some of the ovules had hardly grown at all. The proportion of ovules which fail at this time varies considerably and may reach 50 per cent or even higher. The number which survives presumably bears a close relation to the number of beans in the mature pod. POUND points out that the ovules which fail do so either because they have not been fertilized or because the union is such that the resulting zygote dies. POUND (1931 b) also found a marked variation in bean number in mature pods. In POUND's material the frequency curves of bean number per pod also showed a marked asymmetry, the deviations below the modal number being far more numerous than those above. This suggested incomplete development of potential seeds and led to enquiry into the variability of ovule number per ovary. This number was found to be much less variable and on an average higher than the bean number per pod. This result is evidently in perfect agreement with the conclusion drawn from my own observations, viz. that the variation in bean number is largely due to incomplete pollination. A contributing cause may be inviability of certain zygotic combinations. The latter possibility might occur if a flower happens to be pollinated with a mixture of pollen, part of which gives compatible zygotic combinations, the other part incompatible combinations.

The whole question of seed setting and cherelle wilting in cacao must be considered in the light of similar problems in orchard plants. A review of the observations gathered on the causes of fruit dropping in apples, pears, plums and other orchard plants is given by BRINK and COOPER (1941) in a paper on incomplete seed failure as a result of somatoplastic sterility. In this review it is pointed out that in some cases at least the first fruit-drops represent fruits with unpollinated ovules, the later drops fruits in which embryo development has ceased or is abnormal. BRINK and COOPER point out that this failure probably represents cases of somatoplastic sterility, i. e. a disturbed balance between the endosperm and the maternal tissues.

That aphides contribute to the pollination of the cacao flowers has already been shown by HARLAND (1925) and STAHEL (1928), who observed the amount of pod setting in the presence or absence of various insects. STAHEL concludes that pollination is accomplished by ants which tend the aphides and also, apparently, by various small flying

insects, since wind pollination apparently does not occur. POSNETTE (1944) reviews the whole problem of cacao pollination, especially with reference to the conditions in Trinidad. According to this author, the most important contribution to the extensive literature on the pollination of cacao flowers was made by BILLES (1941) when he drew attention to the Ceratopogonid midge, *Forcipomyia* sp. Subsequent investigation has shown that this insect is probably the most important pollinator and therefore directly responsible for the bulk of the cacao crop in Trinidad.

At the hacienda Clementina in Ecuador I did not observe any larger flying insects in the cacao flowers than thrips. The results described above demonstrate that the aphides, directly or indirectly, through the action of the accompanying ants, contribute to the pollination. This pollination is probably of value for the self-compatible trees and may help to increase the amount of seed setting in such individuals as are now known also to occur in Ecuadorian cacao populations. For seed setting in the self-incompatible trees, on the contrary, the aphides are probably of no value, unless the tending ants occasionally run from one tree to another.

The problem of self-incompatibility in cacao is especially interesting from a theoretical point of view, as incompatibility in this species is not due to a checked pollen tube growth but to a disturbed development after fertilization. Another problem is represented by the fact that in Trinidad self-incompatible trees are also incompatible with each other and only set seed when receiving pollen from a self-compatible tree. In other parts of South America, especially the Amazon region, self-incompatible trees may be compatible with each other. Gradations in the degree of compatibility and incompatibility have also been observed. Incompatibility seems to be more pronounced between individuals within a population than between individuals belonging to different populations. All these facts have chiefly been worked out by the cacao specialists at the Imperial College of Tropical Agriculture, Trinidad, British W. I., and the literature on this problem is already extensive. References to the various publications on this subject by POUND, VOELCKER, COPE, POSNETTE and others are given in a recent paper by POSNETTE (1945). This paper is also of special interest as it deals with pollination results obtained with Amazon material, including material introduced from Ecuador. All plants of the latter kind were found to be self-incompatible, but some combinations between self-incompatibles gave a positive result whereas other combinations failed. The Ecuadorian types were similar in this respect to the other material from the

Amazon region and differed from the Trinitario cacao, in which self-incompatibles cannot intercross successfully.

In my own crosses similar results were obtained, apparently not all self-incompatible trees being incompatible with each other. In contrast to the Ecuadorian types studied by POSNETTE my own material also included a self-fertile tree. Such trees are highly important not only for the seed setting of the self-incompatible trees but also on account of their yield, which, on an average, is higher than the yield of self-incompatible trees. Now that the breeding of new disease-resistant and high-yielding cacao varieties must be based on clonal reproduction (cf. CHEESMAN, 1946), it is necessary to have a detailed knowledge of the conditions of fruit setting in these clones. More basic research is evidently needed before the conditions of pollination and seed production in cacao can be fully understood and controlled.

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# STUDIES ON LINKAGE RELATIONS OF THE $C_y$ FACTORS IN *PISUM*

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## INTRODUCTION.

BESIDES the major factor *Le* for long internodes in *Pisum sativum* L. a number of modifiers are known. Among these the author (LAMM, 1937) has especially studied the polygenic factors  $Cy_1$  and  $Cy_2$ . The  $Cy$  factors are suppressors, which as dominant to a certain degree inhibit the growth in length of the internodes of dwarf peas, i. e. peas recessive for *le*. Of  $Cy_1$  only one recessive allele is known, whereas the locus  $Cy_2$  appears to carry the two mutant genes  $cy_2^c$  and  $cy_2^s$ . Plants of the genotype *le le cy<sub>1</sub>cy<sub>1</sub> cy<sub>2</sub><sup>c</sup>cy<sub>2</sub><sup>c</sup>* are phenotypically so-called crypto-dwarfs, which more especially at the juvenile stage have longer internodes than the common dwarfs. In the slender type (*le le cy<sub>1</sub>cy<sub>1</sub> cy<sub>2</sub><sup>s</sup>cy<sub>2</sub><sup>s</sup>*) the internodal length at any stage is much more elongated than in common dwarfs and crypto-dwarfs. More complete descriptions of these types have been given by RASMUSSEN (1927) and DE HAAN (1927, 1930). In the literature the following symbols have been used for the  $Cy$  factors.

RASMUSSEN	DE HAAN	LAMM	V. ROSEN
1927	1927, 1930	1937	1944
<i>Cry<sub>1</sub></i>	<i>La</i>	<i>Cy<sub>1</sub></i>	—
<i>cry<sub>1</sub></i>	<i>la</i>	<i>cy<sub>1</sub></i>	—
<i>Cry<sub>2</sub></i>	<i>Lb</i>	<i>Cy<sub>2</sub></i>	—
<i>cry<sub>2</sub></i>	—	<i>cy<sub>2</sub><sup>c</sup></i>	—
—	<i>lb</i>	<i>cy<sub>2</sub><sup>s</sup></i>	<i>lb<sup>s</sup></i>

The paper of RASMUSSEN mentioned above was published somewhat earlier in 1927 than that of DE HAAN. In order to co-ordinate the symbols for this group of length factors I decided (LAMM, 1937) to call them »*cy*» with indices. The scope of my recent work has been to find out their linkage relations. I have also tried to find evidence for the correctness of DE HAAN's suggestion of the possible existence of a duplication in *Pisum* (DE HAAN, 1932).

## MATERIAL.

Accurate information as to the origin and genotype of the pure lines used in *Pisum* investigations are highly desirable. Since structural hybridity is probably not rare, it might be of importance to know the relations between the lines used by various authors. A survey of the lines used for the present investigation is given in Table 1.

## RESULTS OF CROSSES AND THEIR STATISTICAL ANALYSIS.

In order to study the linkage relations of the *Cy* factors a number of crosses have been performed. Table 2 gives a survey of the bifactorial segregation numbers collected from  $F_2$  counts employed in the linkage studies. The five first columns of this table contain some general information such as the parental lines, the factors concerned, the phase of crossing (C = coupling, R = repulsion), and the percentage of seed germinated. In this connection it should be added that the pollen-fertility of the  $F_1$  plants has regularly been investigated. No case of total or partial male sterility has been detected. In the next five columns of Table 2 the total number of plants and the observed number of plants of different categories per sixteen are given. If desired, the reader may thus readily compute the absolute number of plants of various categories and also recalculate the  $\chi^2$  analysis given in the following columns of the table. This  $\chi^2$  analysis has been performed in accordance with methods given by MATHER (1938, Chapter IV). In cases of significant single factor deviation,  $\chi^2$  for linkage has been computed according to the special formula given by MATHER, 1938, p. 89. The heterogeneity between crosses and reciprocals has also been computed. With but one exception no significant heterogeneity has been revealed, and therefore  $\chi^2$  values for heterogeneity have not been given in Table 2. Significance has been marked with asterisks, one, two and three asterisks corresponding to respectively  $P = 0,05-0,01$ ,  $P = 0,01-0,001$  and  $P < 0,001$ . Having detected the presence of linkage for two genes further estimations have been made according to the method of maximum likelihood (cf. MATHER, 1938, Chapter V).

### THE LINKAGE RELATIONS OF *Cy*<sub>1</sub>.

The linkage between *Cy*<sub>1</sub> and *St* has been studied in the crosses 43/1, 43/2 and 43/6 of Table 2.  $\chi^2$  for linkage is highly significant. In case of absolute linkage these crosses performed in the repulsion phase

TABLE 1. Survey of the pure lines used for the present investigation.

Line No.	Name (within parenthesis, see footnote)	Origin and references	Marker genes used by the author					
			Stem	Leaf	Flowers	Pod	Seed	
1	Frühe niedrige .....	NILSSON, 1936, L. 76 .....	<i>lc</i>	<i>Wa</i>	<i>Cyl<sup>s</sup></i>	<i>a</i>	<i>N</i>	<i>R</i>
2	Graue niedrige .....	RASMUSSEN, 1927, <i>Gd</i> .....	»	»	»	<i>A</i>	»	»
3	201. 1 .....	DE HAAN, 1927 .....	»	»	»	»	»	»
4	Witham wonder .....	RASMUSSEN, 1927, <i>Ww</i> .....	»	»	<i>Cyl<sup>s</sup></i>	<i>a</i>	»	<i>r</i>
5	English wonder mut. ....	NILSSON, 1933, L. 89 .....	»	»	»	»	»	»
6	204. 1 .....	DE HAAN, 1927 .....	»	»	<i>Cyl<sup>s</sup></i>	<i>wa</i>	»	<i>R</i>
7	Acacia from Vilmorin .....	NILSSON, 1933, L. 54 .....	»	»	»	<i>Wa</i>	»	<i>r</i>
8e	Crypto-dwarf (from 2 × 4) ..	RASMUSSEN, 1927 .....	»	»	<i>Cyl<sup>s</sup></i>	»	»	<i>i</i>
9a	» (from 1 × 5) .....	NILSSON, unpubl. ....	<i>Cyl<sup>s</sup></i>	»	»	<i>a</i>	»	<i>R</i>
9b	» (from 1 × 5) .....	» .....	»	»	»	»	»	<i>r</i>
10	Slender (from 3 × 6) .....	DE HAAN, 1927 .....	»	»	»	»	»	»
11	» (from 7 × 1) .....	NILSSON, 1934 .....	»	<i>Wlo</i>	<i>Cyl<sup>s</sup></i>	<i>A</i>	»	<i>R</i>
13a	» (from 9a × 6) .....	LAMM, 1937, cross 43 .....	»	»	»	<i>a</i>	»	»
13b	» (from 9a × 6) .....	» .....	»	<i>wlo</i>	»	»	»	»
14b	» (from 9a × 11) .....	» .....	»	»	»	»	»	<i>r</i>
15	» (from 2 × 10) .....	» .....	»	»	»	»	»	<i>R</i>
16a	» (from 13a × 22) .....	» .....	»	<i>Wlo</i>	»	<i>A</i>	»	»
16c	» (from 13a × 22) .....	» .....	»	<i>wlo</i>	»	<i>a</i>	»	<i>r</i>
18	Witham wonder mut. ....	HYLMÖ, unpubl. ....	»	»	»	»	»	<i>R</i>
22	Olympia Ws/41 .....	» .....	<i>Cyl<sup>s</sup></i>	<i>Wlo</i>	»	»	<i>N</i>	<i>i</i>
25c	Dwarf (from 18 × 41) .....	LAMM, unpubl. ....	»	»	»	»	»	»
30	» (from 48 × 14b) .....	» .....	»	<i>wb</i>	<i>Cyl<sup>s</sup></i>	<i>b</i>	<i>N</i>	»
40a	Emerald gem .....	NILSSON, 1933, L. 43 .....	<i>Le</i>	<i>Wb</i>	»	»	»	»
41	Chenille-a .....	» .....	»	<i>wlo</i>	»	»	»	<i>R</i>
42	Chenille-A .....	» .....	»	<i>Wlo</i>	»	»	»	»
44	Goldfährnchen .....	» .....	»	<i>wa</i>	»	»	»	»
48	De Winton .....	V. ROSEN, 1944, No. X .....	»	<i>Wa</i>	»	<i>A</i>	»	<i>R</i>
			»	»	»	<i>a</i>	»	<i>gp</i>
			<i>Cyl<sup>s</sup></i>	<i>wb</i>	»	<i>b</i>	<i>A</i>	<i>gp</i>

The pure lines from which the crosses in question have been obtained are given within the parenthesis. These pure lines can be found in the first column of the table. The names of the makers of these crosses are given in the column headed "Origin and references".

TABLE 2. *F<sub>2</sub> analysis of bifactorial crosses.*

Cross No.	Parental lines	Factors	Phase	% germ.	Total No. of plants	Observed number of plants per 16				$\chi^2$ first factor	$\chi^2$ second factor	$\chi^2$ linkage
						AB	Ab	aB	ab			
43/1 .....	30 × 13a	<i>Cy<sub>1</sub>Sl</i>	<i>R</i>	94	163	7.56	4.71	3.73	—	0.247	1.720	22.332***
43/2 .....	"	"	"	98	130	8.98	3.82	3.20	—	1.733	0.092	8.209***
Pooled .....	"	"	"	97	293	8.19	4.31	3.49	—	1.557	0.602	29.519***
43/6 .....	16a × 30	"	"	98	488	7.34	4.56	4.10	—	0.098	3.158	73.457***
Total .....		"	"	98	781	7.66	4.47	3.87	—	0.267	3.534	102.064***
42/3 .....	25c × 15	<i>Cy<sub>1</sub>B</i>	<i>R</i>	99	151	8.69	4.13	2.75	0.42	2.421	0.974	4.363*
40/7 .....	13b × 1	<i>Cy<sub>2</sub>Wa</i>	<i>C</i>	91	157	12.23	0.10	0.20	3.46	0.359	0.614	123.064***
37/17 .....	9b × 2	<i>Cy<sub>2</sub>W/o</i>	<i>C</i>	91	440	8.84	2.84	3.27	1.05	0.982	0.109	0.000
38/4 .....	5 × 6	<i>Wa W/o</i>	<i>R</i>	92	479	8.78	2.97	3.11	1.14	0.585	0.118	0.123
33/34 .....	11 × 4	<i>Cy<sub>2</sub>R</i>	<i>R</i>	95	125	8.96	3.58	2.56	0.90	0.771	0.600	0.108
33/34 .....	11 × 4	<i>Cy<sub>2</sub>I</i>	<i>R</i>	95	125	9.47	3.07	2.69	0.77	0.771	0.067	0.044
33/54 .....	9b × 11	"	"	90	166	8.77	3.47	2.80	0.96	0.201	0.651	0.131
Total .....		"	"	92	291	9.07	3.30	2.75	0.88	0.835	0.194	0.163
33/7 .....	8c × 1	<i>Cy<sub>2</sub>A</i>	<i>R</i>	97	220	8.65	3.49	2.47	1.38	0.097	3.491	0.978
33/9 .....	10 × 1	"	"	98	162	9.38	2.77	3.36	0.49	0.074	1.852	1.451
Total .....		"	"	97	382	8.96	3.18	2.85	1.01	0.171	0.283	0.001
37/18 .....	9a × 2	<i>Cy<sub>2</sub>A</i>	<i>C</i>	99	160	9.70	2.80	2.70	0.80	0.833	0.533	0.011
37/19 .....	11 × 2	"	"	99	283	8.82	2.54	3.11	1.53	2.385	0.029	3.398
Total .....		"	"	99	443	9.14	2.64	2.96	1.26	0.470	0.091	2.681
42/11 .....	16c × 2	<i>Cy<sub>2</sub>N</i>	<i>C</i>	76	100	10.24	3.04	2.08	0.64	3.413	0.213	0.003
42/15 .....	2 × 16c	"	"	72	234	10.19	3.69	1.57	0.55	17.236***	0.279	0.009
Total .....		"	"	74	334	10.20	3.50	1.72	0.55	20.124***	0.036	0.006
40/5 .....	22 × 6	<i>WaN</i>	<i>R</i>	87	146	9.97	2.30	3.29	0.44	0.298	4.831*	0.897
44/79 .....	6 × 44	<i>Wa Gp</i>	<i>R</i>	62	90	8.18	4.09	3.20	0.53	0.133	0.726	3.086
44/84 .....	44 × 6	"	"	73	106	8.91	2.72	3.62	0.75	0.314	0.616	0.507
Total .....		"	"	68	196	8.57	3.35	3.43	0.65	0.027	0.000	2.939

would give the segregation 2 : 1 : 1 : 0. The  $\chi^2$  analysis actually shows a very good agreement with this assumption, and the following non-significant  $\chi^2$  values, each having two degrees of freedom, have been obtained:  $\chi^2$  for deviation from expectation = 3,5481; for differences between reciprocals = 2,2370 and for heterogeneity between crosses = 2,3747. In this case of strong linkage detected in repulsion crosses it would, however, be unwise not to extend the analysis to the  $F_3$ .

For the  $F_3$  analysis seed was taken from the dwarf  $F_2$  plants with normal stipules (category AB of Table 2). Slender  $F_2$  plants were less suitable because of a somewhat reduced seed setting. As shown in Table 3, no fewer than 374  $F_3$  families were investigated. In order to save space the maximum number of seed sown per family was restricted to 60. Altogether 59 families, each giving less than 16 plants, were rejected from the calculations. With this restriction of family size misleading results due to faulty classification should not occur in more than one per cent of cases (cf. MATHER, 1938, pp. 26—27) provided absolute linkage and non-disturbed segregations also characterized these families. In these remaining 315 families of more than sixteen plants germination varied between 27 and 100 per cent, and was especially low in the  $F_3$  progenies of the cross No. 43/6. These circumstances have also been taken into consideration. Obviously segregation values must be cautiously judged in case of bad germination, since there may exist correlation between the germinability and the occurrence of certain of the segregating genotypes. The 2 : 1 : 1 segregating  $F_3$  families (category »m» of Table 3) were chosen for such an investigation, the result of which has been given in the analysis following Table 3.

TABLE 3. *Investigation of  $F_3$  families derived from  $F_2$  plants of the AB type.*

Symbols $F_2$ genotype	Nos. of fam. (> 16) classified into the categories below					Sum <i>n</i>	Not classified (< 16)	Total No. of fam.
	<i>i</i>	<i>j</i>	<i>k</i>	<i>l</i>	<i>m</i>			
	<i>Cy<sub>1</sub>St</i> » »	<i>Cy<sub>1</sub>St</i> <i>cy<sub>1</sub></i> »	<i>Cy<sub>1</sub>St</i> » <i>st</i>	<i>Cy<sub>1</sub>St</i> <i>cy<sub>1</sub>st</i>	<i>Cy<sub>1</sub>st</i> <i>cy<sub>1</sub>St</i>			
Cross No. 43/1 ...	—	2	1	—	72	75	2	77
» » 43/2...	—	3	2	1	63	69	4	73
» » 43/6...	—	4	4	—	163	171	53	224
Observed total	—	9	7	1	298	315	59	374
Exp. $p = 0,0282$	0,1	8,6	8,6	0,3	297,4	315,0		

*Analysis of certain possible correlations in the »m» families of Table 3.* — These families are characterized by 2 : 1 : 1 segregation into plants of the types AB, Ab and aB.

Cross No.	43/1	43/2	43/6
Nos. of families .....	72	63	163
Mean number of plants per family .....	42,6	50,5	34,5
Average percentage of germination .....	72,4	86,5	59,9
» » » <i>Cy<sub>1</sub> st</i> plants .....	26,8	25,0	24,4
» » » <i>cy<sub>1</sub> St</i> » .....	24,2	24,1	26,8
Correlation coeff. (r) between:			
% germinated and % <i>Cy<sub>1</sub> st</i> .....	— 0,192	— 0,016	+ 0,147
» » » » <i>cy<sub>1</sub> St</i> .....	— 0,140	+ 0,133	— 0,087

None of these correlation coefficients are significant.

The above analysis indicates that in spite of the relatively poor germination the risk of misleading classification of the  $F_3$  families is probably fairly small. The occurrence of families of the categories  $j$ ,  $k$  and  $l$  in Table 3 shows that the linkage between  $Cy_1$  and  $St$  is not absolute. The ratio between such 3 : 1 segregating families and the 2 : 1 : 1 segregating families of the  $m$  category has been analysed by the  $\chi^2$  method, with the result that no heterogeneity between crosses could be detected ( $\chi^2$  for heterogeneity = 1,93). For the estimation of the linkage between  $Cy_1$  and  $St$  I have therefore used the observed totals of Table 3. The method of maximum likelihood has also been applied here (cf. MATHER, 1936). Combination of the  $F_2$  and  $F_3$  data discussed in the pages just cited has been avoided, since the present case is that of a recombination value from a close repulsion  $F_2$  (cf. MATHER, 1935, p. 401).

The recombination fraction  $p$  is given by maximizing the logarithm of the likelihood expression

$$\frac{dL}{dp} = i \log \frac{p^2}{2+p^2} + (j+k) \log \frac{2p(1-p)}{2+p^2} + l \log \frac{2p^2}{2+p^2} + m \frac{2(1-p)^2}{2+p^2},$$

which gives

$$2(2i+j+k+2l) - 4np - (j+k+2m)p^2 = 0.$$

Inserting the total values for  $i$ ,  $j$ ,  $k$ ,  $l$ ,  $m$  and  $n$  of Table 3, the solution of the above equation gives  $p = 0,0232$ . The standard error of  $p$  is obtained from the expression

$$sp = \sqrt{\frac{p(1-p)(2+p^2)^2}{4n(2+2p-p^2)}} = \pm 0,006508.$$

The crossing-over value between *Cy*<sub>1</sub> and *St* has thus been estimated at  $2.82 \pm 0.65$  per cent.

It is now easy to calculate the expected segregation of the 781 *F*<sub>2</sub> plants representing the totals for crosses Nos. 43/1, 43/2 and 43/6 of Table 2. A comparison between expected and observed is given below.

Categories	AB	Ab	aB	ab	n
Observed . . . . .	374	218	189	0	781
Expected ( $p = 0.0282$ ) . . . . .	390.7	195.1	195.1	0.2	781.1

$\chi^2$  for deviation (categories aB and ab being pooled) amounts to the non-significant value 3.605.

Table 3 shows that 298 families of the 2 : 1 : 1 segregating category »*m*» have been investigated. These families were represented by no fewer than 11874 plants. It seems perhaps astonishing that not a single slender plant with reduced stipules (*cy*<sub>1</sub> *st*) has been observed among these plants. To answer this question we may calculate the minimum size of family required in order to obtain at least one individual of that category. Applying the detailed instructions given by MATHER (1938, pp. 26—29) with the maximum error 1 : 100 such a family should comprise about 22400 plants. If for some reasons crosses for the detection of linkage can only be made in the repulsion phase, the inference of absolute linkage must obviously be drawn with the greatest caution.

In his extensive investigations of linkage relations in *Pisum* LAMPRECHT (1946) found the average linkage value 27 per cent between *B* and *St*. Cross No. 42/3 of Table 2 shows that there is also, as should be expected, linkage between *Cy*<sub>1</sub> and *B*. The percentage of crossing-over amounts to  $33.9 \pm 7.10$ . Because of the high standard errors, the sequence of the genes *Cy*<sub>1</sub>—*St*—*B* cannot be told with accuracy. Now plants are available, however, making it possible to perform new tests in the coupling phase.

Cross No. 42/3 was made between line No. 25 c and line No. 15. The complete *F*<sub>2</sub> segregation is given in the survey below, where the expected segregations as regards internodal length and flower colour are also inserted.

	Dwarf	Crypto-dwarf	Slender	Total	Expected (9:3:4)
Red . . . . .	82	19	7	108	123.75
Pink . . . . .	39	2	2	43	41.25
White . . . . .	54	11	4	69	55.00
Total	175	32	13	220	—
Expected (12 : 3 : 1)	165.00	41.25	13.75	—	220.00

In neither case is the deviation from expectation significant ( $\chi^2 = 5.643$  and  $2.721$  respectively). For the estimation of the linkage between  $Cy_1$  and  $B$  crypto-dwarf and slender plants have been pooled and white plants have been excluded. The data concerning cross No. 42/3 thus obtained are given in Table 2.

### THE LINKAGE RELATIONS OF $Cy_3$ .

DE HAAN (1927, 1930) found absolute linkage between the length factor  $Lb$  (similar to  $Cy_2$ ) and a factor  $W$  for the production and distribution of bloom. The recessive alleles of these factors were introduced into his crosses by the pure line No. 6. Now this line has further been reciprocally crossed by the author with line No. 40 a, which is recessive for the known wax-factor  $Wa$ . The  $F_1$  and  $F_2$  of these crosses were constantly emerald (non-waxy). The number of  $F_2$  plants investigated was 147 in the cross No. 39/5 between lines Nos.  $6 \times 40$  a and 463 in the reciprocal cross No. 39/6. The germinability was in both cases good, being 91 and 95 per cent respectively. These results show that the wax-factor of line No. 6 is identical with  $wa$ .

Provided the plants are dominant for the basic wax-gene  $Bl$  a necessary condition for the normal glaucous covering of their green parts is dominance in either  $Wa$ ,  $Wb$  or in both of them (WELLENSIEK, 1928). This author has shown that recessivity of these genes results in a more or less complete reduction of the bloom, giving plants of emerald type. Of either gene WELLENSIEK claims to have found three multiple allelomorphs. His descriptions of the different genotypes are unfortunately somewhat incomplete, as pointed out by LAMPRECHT (1939), who gives good descriptions for the distribution of wax characterizing the plants of his own lines.

As regards the symbols for these wax-genes, I have followed the modifications introduced by NILSSON (1933), which have also been adopted by LAMPRECHT (1939). In the present investigation no attempts have been made to distinguish between the recessive allelomorphs within the  $Wa$ - and  $Wb$ -series. Line No. 6 as well as line No. 40 a were characterized by slightly glaucous stems in contrast to the non-waxy stipules and pods. The leaflets of the former line appeared to be more glaucous than those of the latter. WELLENSIEK (1928), however, points out that the amount of wax on the foliage does not demonstrate typical differences. In a cross between line No. 40 a and line No. 42 a beautiful 9:7 segregation was obtained with respect to the presence of bloom. This cross was made in order to control the genotype of the former line, and it verified a similar result earlier obtained by NILSSON (1933, p. 220). Line No. 42 was also crossed with line No. 6. As expected, the  $F_1$  was glaucous and the  $F_2$  segregated into glaucous and emerald. Unfortunately the proportions were not determined.

As mentioned earlier, DE HAAN considered the linkage between  $Cy_2$  and the wax-factor, now known to be  $Wa$ , to be absolute. The results

of the particular crosses upon which this conclusion was based are given in Table 4, where a control cross made by the author is also inserted. In case of free combination the four categories of this table would have occurred in the ratio 45 : 15 : 3 : 1. The observed segregations are, however, in accordance with that expected for absolute linkage, i. e. 48 : 12 : 0 : 4 with the exception of the results obtained by DE HAAN (1927), where excess of dwarf, emerald plants contributes to a significant  $\chi^2$ . In spite of this, neither the total deviation nor the heterogeneity is significant. In this connection it is perhaps of some interest

TABLE 4.  $F_2$  analysis of crosses between line 6 and 3.  
( $Cy_1cy_2s_wa \times cy_1Cy_2Wa.$ )

Results obtained by	Number of plants classified as:				Total No. of plants	$\chi^2$ for dev.
	dwarf glaucous	dwarf emerald	slender glaucous	slender emerald		
DE HAAN, 1927.....	423	137	—	39	599	7,026*
» » , 1930.....	5426	1417	—	439	7282	2,754
LAMM, 1942.....	320	79	—	21	420	1,130
Total observed.....	6169	1633	—	499	8301	
Exp. 48 : 12 : 0 : 4 ( $p = 0$ )	6225,8	1556,4	—	518,8	8301,0	
$\chi^2$ for deviation.....	0,517	3,766	—	0,757		5,040
» » heterog. ....						5,870
Exp. $p = 0,0199$ .....	6205,3	1576,9	20,44	498,4	8301,0	
$\chi^2$ for deviation.....	0,212	1,997	20,440	0,001		22,650***

to point out that in progenies segregating for *Wb* the recessives, according to v. ROSEN (1944, p. 272), are in excess.

The linkage between  $Cy_2$  and *Wa* has also been studied in cross No. 40/7 (of Table 2) performed between the slender line 13 b and the dwarf line No. 1. This slender line in turn has been extracted from the progeny of a cross between lines 9 a and 6. The recessives are thus of the same origin as in the crosses of DE HAAN, though the genotypical background is different. Quite against the assumption of absolute linkage three recombination plants appeared among 157  $F_2$  plants. The crossing-over percentage as determined by the method of maximum likelihood amounts to  $1,99 \pm 1,13$ . The two bottom rows of Table 4 show that the results of cross No. 40/7 are significantly different from the cases of seemingly absolute linkage previously discussed. If this disagreement is due to real differences in the frequencies of crossing-over (i. e. genetically changed linkage values) or if they are caused by

differences in the viability of certain genotypes or other circumstances cannot be determined from the present investigations. Anyhow, the earlier assumption of absolute linkage between  $Cy_2$  and  $Wa$  must not be generalized.

In this connection it should also be pointed out that the description of the slender phenotype given by DE HAAN (1930) in certain details is only valid for his own lines. This author observed a rather reduced fertility with regard to the seed content of the pods. Frequently, seedless pods were formed. Among slender lines of other origin, e. g. among those which I obtained from NILSSON (cf. LAMM, 1937), seed setting was much more abundant although still reduced as compared with normal dwarfs. It does not seem quite impossible that there might be an interaction between the viability of certain genotypes and the degree of seed setting.

In my earlier paper (LAMM, 1937) I suggested that  $Cy_2$  was linked with  $Wlo$  (for the description of this gene, see NILSSON, 1933). Unfortunately this was definitely a mistake due to insufficient knowledge of the material at that time. The slender plants were studied at a rather young stage, and their relatively tender leaflets were misclassified as belonging to the  $wlo$  type. More recent studies have shown that there is no indication of linkage between  $Cy_2$  and  $Wlo$  or  $Wa$  and  $Wlo$ , as can be seen from cross Nos. 37/17 and 38/4 of Table 2.

In the  $F_2$  progeny of the cross last mentioned an unexpected segregation of the length of the young plants was observed. The same phenomenon of an unexpected length segregation has been noted in crosses between lines 6 and 18. That other factors than  $Cy_1$  and  $Cy_2$  also modify the length of dwarf peas is known from the investigations of DE HAAN (1930) and RASMUSSEN (1938).

RASMUSSEN (1927) and DE HAAN (1927, 1930) have shown independent segregation between  $Cy_2$  and the two length factors  $Cy_1$  and  $Le$ . WELLENSIEK (1928) states that there is no linkage between  $Wa$ ,  $Wb$ ,  $R$  and  $I$ . Since there is strong linkage between  $Cy_2$  and  $Wa$ , the former factor cannot be coupled with any one of the three factors last mentioned. With respect to  $R$  and  $I$  the crosses Nos. 33/34 and 33/54 of Table 2 further support this statement. As regards the cross No. 33/34 segregating into 12 dwarf : 3 crypto-dwarf : 1 slender the linkage relations were determined within the two categories last mentioned.

In studies of small progenies v. ROSEN (1944, p. 300) found indications of independent segregation between  $Cy_2$  and the genes  $D$ ,  $M$  and  $A$ , whereas a possible linkage of 42 per cent might exist between  $Cy_2$  and  $Td$ . He strongly points out that this rather unsure linkage relation must not be taken for granted. The crosses 33/7, 33/9, 37/18

and 37/19 of Table 2 are all concerned with the linkage relation between  $Cy_2$  and  $A$ . A significant heterogeneity ( $\chi^2 = 5.060$ ) has been found between the second factor segregation of the crosses Nos. 33/7 and 33/9. Estimations based on the totals of these two crosses therefore have little value and perhaps ought to have been omitted from the table. In the cross 37/19 there is a slight indication of linkage between  $Cy_2$  and  $A$ , but there is no significance, and my own results together with those obtained by v. ROSEN (1944) support the statement of independent segregation.

In 15:1 segregating crosses with respect to dwarf and slender or crypto-dwarf, which crosses were at the same time heterozygous for  $A$ , the proportion of red: white among the crypto-dwarf and slender plants was 50:19. The expected proportion in case of independent segregation between  $Cy_2$  and  $A$  is 51.75:17.25.

$Td$  by LAMPRECHT (1945) was localized to a linkage group of the constitution  $N-15\% - Z-18\% - Fa-28\% - Td$ . If v. ROSEN's suggestion of a linkage between  $Cy_2$  and  $Td$  corresponding to 42 per cent crossing-over is true, the former gene if situated to the left of  $Td$  must show linkage with  $N$ . The crosses 42/11, 42/15 and 40/5 of Table 2 refute this assumption. Two of these crosses are characterized by bad single factor segregations, but in spite of this the statement of independent segregation between  $Cy_2$  and  $N$  seems to be valid. On the other hand, should  $Cy_2$  happen to be situated to the right of  $Td$  the possible linkage between these two genes is still an open question.

The last but most interesting linkage relation to be discussed is that between  $Wa$  and  $Gp$  investigated by means of the two small progenies of the crosses 44/79 and 44/84 of Table 2. Unfortunately the germination power of the seed was bad, which contributes to the insecurity of the results. There is no heterogeneity between the two crosses. The single factor segregations of their totals are good, whereas  $\chi^2$  for linkage is somewhat high but not at all significant. In spite of this, linkage has been calculated by the method of maximum likelihood. The crossing-over value for the total of the two crosses thus obtained amounts to  $39.9 \pm 5.92$  per cent.

I also tried to get a yellow-podded slender line in order to be able to test the linkage between  $Cy_2$  and  $Gp$  in the coupling phase by crossing line No. 44 with the slender line No. 14 b. DE HAAN (1930) in crosses between high and slender obtained the segregation 45 high:15 dwarf:4 slender. Of cross No. 41/5 (line 44  $\times$  14 b) 343  $F_2$  seeds were obtained, while 345 seeds resulted from the reciprocal cross No. 41/6. These seeds were sown, and respectively 21 and 14 slender plants were

obtained, all of which were rather weak and did not produce pods. The progenies also contained high and dwarf plants. This at present inexplicable behaviour of the exceptionally slender plants seems worthy of mention in this connection.

Future investigations of the possible linkage between  $Cy_2$  and the genes of the  $Gp$  group are, however, very much needed. For if the crossing-over value of about 40 per cent obtained between  $Wa$  and  $Gp$  should turn out to be true, this would be attended by some interesting consequences, which will be discussed in the following chapter. The author, however, is anxious that the linkage suggested should not be taken for granted until new facts are available. The linkage of  $Cy_2$  is thus still an open question.

### DISCUSSION.

As mentioned in the introduction, I have tried to prove the existence of a duplication in *Pisum*. If a number of different polygenic (15 : 1 segregating) pairs of genes were distributed between two structurally and genetically similar linkage segments situated in two non-homologous chromosomes, this would be a strong indication of duplication. Genes that might be involved are, for instance,  $Cy_1$  and  $Cy_2$ ,  $F$  and  $Fs$  (cf. WINGE, 1936; LAMPRECHT, 1946) as well as  $Em_1$  and  $Em_2$  (DE HAAN, 1932). In such an investigation 9 : 7 segregating genes, e. g.  $Wa$  and  $Wb$  or  $P$  and  $V$ , should also be taken into consideration, since there is no fundamental differences between duplicate and complementary genes (cf. EYSTER, 1934, pp. 311—312). The possibility of a gradual divergence in the course of evolution between originally identical genes distributed over different members of a repeat (cf. WHITE, 1945, p. 48) should also be accounted for, and an absolutely similar effect of the corresponding genes could not be expected.

It is very likely that  $Cy_1$  is situated between  $F$  and  $St$ . If the assumption of about 40 per cent crossing-over between  $Wa$  and  $Gp$  should turn out to be true,  $Cy_2$  would probably be situated in the neighbourhood of  $Fs$ . According to LAMPRECHT (1942 a),  $Gp$  belongs to a linkage group of the constitution  $Gp-3.2\%-Cp-32.3\%-Fs-<1\%-Ast$ , the percentage of crossing-over between  $Gp$  and  $Fs$  being 37.1. A duplication with segments containing respectively  $F$   $Cy_1$  and  $Fs$   $Cy_2$  would then most probably exist.

LAMPRECHT (1946) from quite different suppositions postulates the existence of small homologous segments in these previously discussed regions of non-homologous chromosomes. In a lecture at the Mendelian

Society, in Lund, in the autumn of 1944 NILSSON (unpubl.) reported that he had detected linkage between a point of translocation and the genes *St B* in lines originating from the variety Extra Rapid. LAMPRECHT (1946) has been able to locate the point of translocation to the segment between *F* and *St* of the *B* chromosome and *Fs* and *Ast* of the *Gp* chromosome. The translocation studied by him is most probably identical with that investigated by NILSSON. LAMPRECHT further suggests that the appearance of semisterile plants, such as frequently occur in *Pisum*, are due to crossing-over between small homologous segments of non-homologous chromosomes. He also claims that the well-known figure of eight in *Pisum* (SANSOME, 1932) supports this duplication hypothesis. It should be pointed out that SANSOME himself is aware that this explanation is an alternative to the one generally accepted. A circumstance of interest in this connection is that the so-called interstitial segment in the figure of eight must be of considerable length and even allow of two chiasmata being formed (SANSOME, 1932). Anyhow, it would be of interest to determine the genes concerned.

Provided *Cy<sub>2</sub>* is really situated in the way suggested a lot of interesting problems will arise. We have not far to look for an explanation of genetically changed linkage values between *Cy<sub>2</sub>* and *Wa* in a possible inversion of the *Cy<sub>2</sub>—Fs* region. The relations between the *Lc* and *Ld* factors described by DE HAAN (1930) and the *Cy* factors ought to be thoroughly studied. If new members of the *Cy* polygenes should thereby be detected, this might be of importance with respect to the duplication hypothesis. Further, it should be of interest to investigate the reasons that *Cy<sub>1</sub>* and *F* are only represented by two alleles while three multiple allelomorphs are known of *Cy<sub>2</sub>* (LAMM, 1937), *Wa* (WELLENSIEK, 1928) and *Fs* (LAMPRECHT, 1942). *Td*, according to LAMPRECHT (1945), is also probably represented by three allelomorphs. As, however, the position of *Cy<sub>2</sub>* is not definitely determined, these speculations are perhaps idle, and are mainly presented in order to emphasize the possible importance of future investigations.

The behaviour of the *Cy* factors is also interesting from the point of view of variation and selection of polygenic characters. I have the impression that dwarfs dominant for either *Cy<sub>1</sub>* or *Cy<sub>2</sub>* are most common, which is in line with the theories of polygenic inheritance given by MATHER (1941). Exact studies of these conditions would, however, be rather difficult and tedious.

I wish to express my gratitude to the State Horticultural Research Station at Alnarp for granting me the loan of experimental grounds and

of manual help, and address these thanks to the Head of the Institute, Professor F. NILSSON. I am also indebted to several colleagues mentioned in this paper for supplying me with material. To many other persons I also wish to express my thanks for valuable assistance and help in various ways.

### SUMMARY.

In the present investigation of the linkage relations of the *Cy* factors in *Pisum sativum* the following main results have been obtained.

(1) The length factor *Cy*<sub>1</sub> is closely linked with *St*.

(2) The length factor *Cy*<sub>2</sub> is closely linked with *Wa*. It gives independent segregation with *A*, *Cy*<sub>1</sub>, *D*, *I*, *Le*, *N*, *Wb* and *Wlo* but may perhaps belong to the *Gp* group.

(3) If *Cy*<sub>2</sub> and *Gp* are coupled in the way assumed in this paper, this would indicate that *Pisum sativum* has a duplication. This must not, however, be taken for granted until actually proved.

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## ABSTRACTS — KURZE MITTEILUNGEN

GUDMUND SMITH: Psychological tests with twins.

On a material of 16 uniovular and 10 binovular pairs of twins of ages between 9 and 44 years (SMITH, 1946) a series of tests was performed to determine how a given objective environment is undergone and moulded by different individuals. Could it be expected that the subjective — and at the same time the significant objective — environment would be the same for all when the objective environment was the same? If this was not the case, would the subjective environments then show more agreements in individuals possessing similar hereditary factors than in those having different factors? As the answering of these questions required a rather intimate knowledge of the persons being tested, the characterological structure of these was determined by means of casuistic studies as well as modified Wartegg tests (WARTEGG, 1929).

In the first section of the test-series the objective environment consisted of a number of incomplete pictures, sketched or coloured, which the individuals under test were asked to interpret. In the second section it consisted of a puzzle with 13 combination possibilities which the subject had to set up in the way he thought most natural. The pictures were taken from O. MÜLLER (1929) and WEIL (1929), the idea of the puzzle from G. H. FISCHER (1944). In many respects the results show so great a variation that the first question can be answered straightaway: each individual selects or forms an individual environment out of the given objective environment.

On comparison of uniovular with binovular twins it was observed that the former agreed more often in their subjective environment than the latter. The interpretations and combinations made by uniovular twins were at times even almost identical. The agreement between them, however, was not found throughout the whole material. Four of the 16 pairs showed distinct discordance in the interpretation tests. In certain details of the picture material all the pairs of twins displayed concordance. But there, the inter-individual variation ceased, and hence no twin discordance is to be expected.

A close analysis of the results shows that the environmental discordance is not an accidental one but corresponds to a discordance in characterological structure. This applies not least of all to uniovular twins, whose individual choice of environment in the tests often appeared as divergences of interest in their natural environment. The question of the ultimate causes of this difference in character pattern — and consequently in environment and development — cannot yet be definitely answered. The working hypothesis must for the present be that a small difference in character — perhaps already induced by intra-uterine influences of environment, or by something else — produces an environmental discordance, which accentuates the difference in character and so on to a constantly increasing extent; as years pass only few pairs retain all their original similarity.

A comparison between various test-results within the pairs is also of interest. The discordance disclosed in the interpretation test by a uniovular pair need not be repeated in the combination test. Discordance may appear

or not, according to the aspect chosen for study and the objective environment into which the pair is introduced. Moreover, all uniovular twins do not show — as is evident from the above — the same differences, such being found to vary with each tested pair.

Some of the statements made on the strength of these test-results may seem rather self-evident, viz. that the discordance between uniovular twins varies from pair to pair and that different twins obtain dissimilar personal and, as we calculate, significant objective environments. There are, however, many scientists who are more or less unwilling to take such everyday observations into due consideration. Both BOUTERWEK (1935) and ECKLE (1939) — the latter more cautiously — generalize discordance in character under the antithesis »firm—loose». And those engaged in the mathematical calculation of the influence of hereditary and environmental factors upon variation often assume the same middle environmental difference for uniovular as well as binovular pairs. This material shows indeed that binovular pairs display a much greater one.

The investigation also rendered it possible to evaluate the specimens employed as to their original quality as test-material for JAENSCH's integration typology. They can scarcely be said to give unambiguous results.

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#### BÖRJE LÖVKVIST: Chromosome studies in *Cardamine*.

The following study began some years ago and was made in order to elucidate the relationships between *Cardamine pratensis* and *C. dentata*.

By means of cytological investigations on several hundred plants of this material collected in nature numerous chromosome races were found. *Cardamine pratensis* and *C. dentata* are probably the extremes in a series of types having more or fewer traits from the one or the other of the two species. As yet it is impossible to say anything about the taxonomy of this species complex.

Investigations of plants from the southern part of Sweden demonstrated a great variation in chromosome number. Somatic studies resulted in the distinction of the following races:  $2n = 30, 56, 60, 64, 68, 72$  and  $76$ . Plants

with 58 and 84 chromosomes were found, but not in so high a frequency that they deserve to be regarded as regular races. Plants with  $2n = 30, 56$  and  $76$  have been studied in meiosis also. Only in those with the lowest number have meiotic irregularities as yet been found.

Plants of different races very often grow together in meadows, but always in a regional distribution, with the lowest number in only higher parts and with the races  $2n = 56, 60, 64$  and  $68$  in lower parts, and the races with  $72$  and  $76$  near or in the water. Some examples can be given here.

Locality	Chromosome number in		
	higher parts	lower parts	near water
Fågelsång . . . . .	30	—, 60, —, 68	72, 76
Kungsmarken . . . . .	30	56, —, —, 68	72, 76
Kalthus . . . . .	30	—, 60, 64, —	—, —
Böljanemosse . . . . .	—	56, 60, 64, 68	—, 76
Revinge . . . . .	30	—, —, —, —	72, —
Värpinge . . . . .	30	56, 60, 64, —	—, —
Revlingemosse . . . . .	—	—, —, 64, —	72, —

The conclusion drawn from this investigation is that in the present case higher chromosome number is correlated to higher water content of the soil. Lund, Institute of Genetics, January, 1947.

ANTERO VAARAMA: Contributions to the cytology of the genus *Berberis*.

In the large genus *Berberis* the dominating chromosome number seems to be  $2n = 28$  (TISCHLER, 1928, 1929; DERMEN, 1931; GIFFEN, 1936). The primary basic number has been supposed to be  $x = 7$ . Consequently the species with 28 chromosomes are tetraploids. Only in two species, *Berberis buxifolia* (TISCHLER, 1928; GIFFEN, 1936) and *B. turcomanica* var. *integrifolia* (DERMEN, 1931), has the chromosome number  $2n = 56$  been counted. In regard to the basic number 7, these are octoploids.

During my stay in the Cyto-genetic Laboratory of Svalöf in the summer of 1945 I had an opportunity to examine some species and hybrids of the group of evergreen barberries in prepared material kindly placed at my disposal by the Head of the Laboratory, Dr. ALBERT LEVAN. The fixation of the root-tips was performed in the Ramlösa Plant Nurseries (Hälsingborg, Sweden), whose Director, Mr. HOLGER JENSEN, had kindly afforded facilities for this work. The *Berberis candidula* material, however, is from the plant nursery of the Alnarp Horticultural Institute (Åkarp, Sweden).

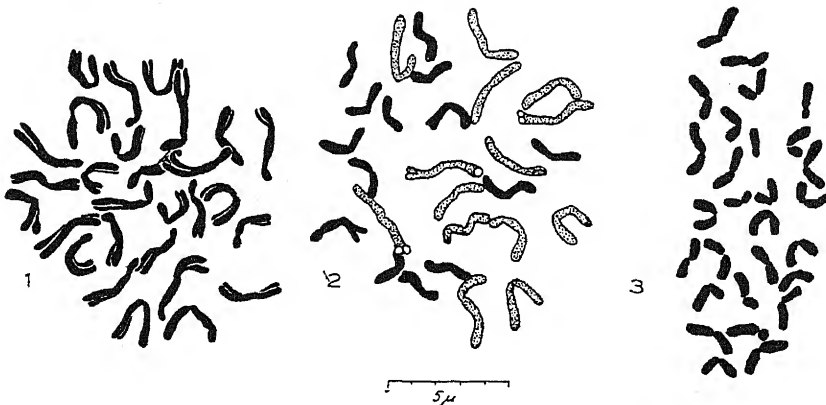
The only fixative used was the craf-modification commonly used in Svalöf. The paraffin sections were stained partly with FEULGEN, partly using the common iodine-gentian-violet method. It was found that the *Berberis* chromosomes are rather hard to stain. To obtain satisfactory results it was necessary to stain for 30 minutes in gentian-violet.

In the root-tip mitoses of the following seven *Berberis* species the chromosome number  $2n = 28$  was counted: *Berberis candidula* SCHNEID., *B. Gagne-*

*painii* SCHNEID., *B. ilicifolia* FORST., *B. Julianae* SCHNEID., *B. Sargentiana* SCHNEID., *B. stenophylla* LINDL., *B. verruculosa* HEMSL. and WILS. All numbers are equal to those counted earlier from the same species (DERMEN, 1931; GIFFEN, 1936).

The number  $2n = 56$  was observed in the following plants: *Berberis buxifolia* var. *nana* SCHNEID. and *B. actinacantha* MART. As mentioned above, the pure species *B. buxifolia* POIR. (TISCHLER, 1928; GIFFEN, 1936) has the same number. The chromosome number of the variety *nana* and the species *B. actinacantha* was not known earlier.

Among the material were also root-tips from the genus hybrid *Mahonia aquifolium*  $\times$  *Berberis candidula* (= *Mahoberberis aquicandidula*), an artificial cross made by Mr. HOLGER JENSEN at Ramlösa. Two other *Mahonia*—*Ber-*



Figs. 1—3. — Fig. 1. *Berberis candidula*:  $2n = 28$ . — Fig. 2. *Mahonia aquifolium*  $\times$  *Berberis candidula*:  $2n = 28$ . The 14 short *Mahonia* chromosomes are black, the 14 long *Berberis* chromosomes are dotted. — Fig. 3. *Mahonia aquifolium*:  $2n = 28$  (from LEVAN, 1944). —  $\times 3000$ .

*beris* crosses were previously known, viz. the old *M. aquifolium*  $\times$  *B. vulgaris* (= *Berberis Neubertii*) and *M. aquifolium*  $\times$  *B. Sargentiana* (LEVAN, 1944), the earlier successful cross of Mr. JENSEN.

It was very interesting to see whether the individual size of the chromosomes as determined by LEVAN (l. c.) in *M. aquifolium*  $\times$  *B. Sargentiana* was also maintained in the present case.

The chromosome number of *Mahoberberis aquicandidula* is  $2n = 28$  and is thus the same as that of the parent species. A closer examination reveals that the chromosomes of *B. candidula* are partly median, partly submedian. The length of the chromosomes varies from 2.5 to 4.2  $\mu$ . Of the chromosome complement two pairs have a length of 4.0—4.2  $\mu$ , four pairs 3.3—3.5  $\mu$ , three pairs 3.0—3.2  $\mu$ , four pairs 2.7—2.8  $\mu$  and one pair 2.5  $\mu$  (Fig. 1).

Even this superficial survey of the chromosome morphology shows that if the haploid chromosome set of *B. candidula*,  $n = 14$ , is composed of two different basic sets of seven, in any case the sets differ from each other. Autotetraploidy is, therefore, out of question.

The chromosome size of *Mahonia aquifolium* varies between 1,5—2,5  $\mu$  (LEVAN, 1944). The length of five pairs is 2,3—2,5  $\mu$ , the others being smaller (Fig. 3).

The chromosomes of the hybrid can be divided on the basis of their length into two distinct groups (Fig. 2, black and dotted chromosomes). Among the group of the 14 longer chromosomes the following lengths can be found: 2 units about 4  $\mu$ , 4 units of 3,5—3,8  $\mu$ , 3 units of 3,2—3,3  $\mu$ , 4 units of 2,7—3,0  $\mu$  and one unit of 2,6  $\mu$ . Among the group of 14 shorter chromosomes there are 5 units of 2,4—2,6  $\mu$ , the others being 2,3—1,8  $\mu$ .

As can be seen, the length-classes of the hybrid correspond very well to those found in the parents. The chromosomes of the hybrid seem, however, to be somewhat longer than those of the parents. This may be due to the differences in fixation, but another possible cause is the different spiralization in the hybrid chromosomes. Anyhow, in the hybrid we are able to distinguish, except for one chromosome, the whole *Berberis* set from the chromosomes of *Mahonia*. The shortest *Berberis* chromosome belongs to the same length-class as five *Mahonia* chromosomes and is therefore impossible to identify.

At the same time as the conclusions of LEVAN (1944) are supported by the present observations, these are in accordance with the view that the individual size of the parent chromosomes is maintained in hybridization. Owing to the non-flowering of the *Mahonia*—*Berberis* hybrids it was not possible to study the properties of the meiosis. In the literature there are to be found many references to the unequal bivalents in analogous hybrids. The question was last treated by MEURMAN and SUOMALAINEN (1946). In their material, the hybrid *Montbretia*, besides unequal bivalents, they also observed unequal partners in the second division of meiosis.

In this connection I wish to present my hearty thanks to Dr. ALBERT LEVAN and Miss MAGNA PALM for all their kind help. I am also greatly indebted to Mr. HOLGER JENSEN and Dr. EMIL JOHANSSON for the material kindly provided by them.

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# PARTHENOGENESE UND POLYPLOIDIE BEI RÜSSELKÄFERN (CURCULIONIDAE)

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(With a Summary in English)

O BWOHL die parthenogenetische Vermehrung bei den Käfern im allgemeinen sehr selten ist, machen die Rüsselkäfer, Curculionidae, in dieser Hinsicht eine Ausnahme. Bei ihnen ist nämlich die Parthenogenese in den Unterfamilien Otiorrhynchinae und Brachyderinae eine verhältnismässig allgemeine Erscheinung. Es ist festgestellt worden (SUOMALAINEN, 1940 a und b), dass die meisten parthenogenetischen Curculionidenarten polyploid sind. Von den vom Verf. (op. c.) untersuchten finnischen Arten erwies sich nur eine (*Polydrosus mollis*) als diploid mit 22 Chromosomen. Fünf Arten (*Otiorrhynchus ovatus*, *O. ligustici*, *Strophosomus melanogrammus*, *Trachyploeus bifoveolatus* und *Sciaphilus asperatus*) sind triploid mit 33 oder einer anderen nahestehenden Zahl von Chromosomen. Drei Arten (*Otiorrhynchus dubius*, *O. scaber* und *Barynotus obscurus*) wiederum haben 44 Chromosomen und sind dadurch tetraploid. Weil die Polyploidie im Tierreich sehr selten ist, verdient diese bei den parthenogenetischen Rüsselkäfern festgestellte polyploide Artenreihe in mancher Hinsicht ein gewisses Interesse.

Um die Parthenogenese und Polyploidie bei den Rüsselkäfern möglichst gut kennen zu lernen und zu verstehen, ist es notwendig, möglichst viele Arten zytologisch zu untersuchen, und zwar sogar in verschiedenen Teilen ihrer betreffenden Verbreitungsgebiete. Man erhält dadurch u. a. die Möglichkeit, einerseits die eventuell vorhandenen fortpflanzungsbiologisch und zytologisch verschiedenen Rassen ein und derselben Art — bzw. entsprechende einander nahestehende Arten — zu finden und ihre Verbreitung kennen zu lernen, anderseits auch die Chromosomenverhältnisse einander entsprechender bisexueller und parthenogenetischer Rassen bzw. Arten miteinander zu vergleichen.

Im folgenden werden die Chromosomenverhältnisse einiger mitteleuropäischen und bisher nicht untersuchten finnischen Curculionidenarten beschrieben. Die meisten hier behandelten Arten gehören der

umfangreichen Gattung *Otiorrhynchus* an, einer Gattung, die gute Möglichkeiten zur Klärung einiger wesentlichen Fragen der Parthenogenese bietet, weil in ihrem Bereich viele einander nahestehende bisexuelle wie auch parthenogenetische Formen anzutreffen sind (Näheres bei APFELBECK, 1928 und 1930; SZÉKESSY, 1937; SUOMALAINEN, 1940 b, S. 21—26; JAHN, 1941).

## I. MATERIAL UND METHODEN.

Die vorliegende Arbeit betrifft die Chromosomenverhältnisse bei insgesamt 20 Curculionidenarten. Von diesen fallen die meisten der Gattung *Otiorrhynchus* zu. Die untersuchten *Otiorrhynchus*-Arten sind die folgenden (System und Nomenklatur nach WINKLER [1932]; die von mir früher [op. c.] zytologisch untersuchten Arten sind in Klammern beigegeben):

	Bisexuell		Parthenogenet.
	Spermatogen.	Oogenese	Oogenese
Untergattung <i>Dodecastichus</i> STIERL.			
* <i>O. inflatus</i> GYLL. ....	+		
<i>O. geniculatus</i> GERM. ....	+	+	
Untergattung <i>Otiorrhynchus</i> s. str.			
<i>O. sensitivus</i> SCOP. ....	+	+	
* <i>O. armadillo</i> ROSSI ....	+	+	
<i>O. bisulcatus</i> F. ....	+	+	
<i>O. niger</i> F. ....	+	+	+
<i>O. fuscipes</i> OL. ....	+		
<i>O. morio</i> F. ....	+	+	
( <i>O. arcticus</i> O. F.) ....	(+)	(+)	
( <i>O. dubius</i> STRÖM) ....			(+)
* <i>O. scaber</i> L. ....			+(+)
Untergattung <i>Dorymerus</i> SEIDL.			
<i>O. austriacus</i> F. ....		+	
* <i>O. equestris</i> RICHT. ....	+		
<i>O. singularis</i> L. ....			+
* <i>O. pupillatus</i> GYLL. ....			+
<i>O. salicis</i> STRÖM ....	+	+	+
<i>O. sulcatus</i> F. ....			+
<i>O. gemmatus</i> SCOP. ....	+	+	+
Untergattung <i>Tournieria</i> STIERL.			
( <i>O. ovatus</i> L.) ....			(+)
Untergattung <i>Arammichnus</i> GOZIS			
( <i>O. ligustici</i> L.) ....			(+)
Untergattung <i>Tyloderes</i> SCHÖNH.			
<i>O. chrysops</i> HBST. ....		+	

Neben den obenangeführten *Otiorrhynchus*-Arten werden die Chromosomenverhältnisse noch bei drei anderen parthenogenetischen Rüsselkäferarten behandelt. Diese sind *Sciaphilus asperatus* BONSD., *Strophosomus melanogrammus* FÖRST. und \**Barynotus moerens* F. Die mit einem Sternchen (\*) bezeichneten Arten hat Herr Professor Dr. K. HOLDHAUS (Wien) bestimmt oder meine Bestimmung bestätigt, wofür ich ihm bestens danke.

Das Untersuchungsmaterial stammt hauptsächlich aus der Umgebung der Biologischen Station Lunz (Lunz am See) in den Österreichischen Kalkalpen. Ein kleinerer Teil desselben ist in der Umgebung von Berlin oder in Helsinki gesammelt worden. Genauere Angaben über den Ursprung und den Umfang des Materials sind im Zusammenhang mit den Artenbesprechungen zu finden.

Zur Untersuchung der Spermatogenese wurden Testes von Imagines in 0,6-prozentiger Normosallösung herauspräpariert. Zur Fixierung und Färbung ist Karminessigsäure verwendet worden (Quetsch-Dauerpräparate nach dem bei BAUER und TIMOFÉEFF-RESSOVSKY [1939] angegebenen Verfahren). Häufig wurden die Präparate mit dem Gemisch von BOUIN-ALLEN (BAUERS Modifikation; Näheres bei BAUER, 1931) nachfixiert und mit fuchsinschweflicher Säure nach FEULGEN nachgefärbt. Wenn mit dem Nukleolus zusammenhängende Fragen zu untersuchen waren, wurde auch Methylgrün oder Eisenhämatoxylin (nach HEIDENHAIN) zur Nachfärbung verwendet.

Die Ovarien und die abgelegten Eier wurden mit CARNOYS Gemisch (6 : 3 : 1) fixiert, weil dieses in die Eier gut und schnell eindringt, und danach über Chloroform in Parovax eingebettet. Sämtliche abgelegten Eier und die schwerer schneidbaren Ovarien wurden jedoch nach der Buthylalkohol-Methode in das Parovax gebracht. Die Dicke der Parovaxschnitte betrug bei den Ovarien sowie bei den abgelegten Eiern 15  $\mu$ . Sie wurden nach FEULGEN gefärbt.

Die Zeichnungen sind mit Hilfe des Objektivs 120  $\times$  (Apochromat), des Okulars 25  $\times$  und des ABBESchen Zeichenapparäts (alles Zeiss) auf der Höhe des Objektisches ausgeführt worden. Die Vergrößerung ist, nachdem die Abbildungen beim Klischieren auf  $\frac{2}{3}$  der ursprünglichen Grösse verkleinert sind, also 2000fach. In einigen Abbildungen wurden die Chromosomen zur besseren Übersicht getrennt gezeichnet oder ungeordnet; das ist in den betreffenden Bildertexten mit dem Zeichen  $\longleftrightarrow$  vermerkt.

In den Bildertexten gelten folgenden Abkürzungen: KES = Fixierung und Färbung mit Karminessigsäure. KES + B—A + Feulg. =

= Fixierung und Färbung mit Karminessigsäure; Nachfixierung mit dem Gemisch von BOUIN-ALLEN und Nachfärbung nach FEULGEN. C + Feulg. = Fixierung mit CARNOYS Gemisch und Färbung nach FEULGEN.

## II. DIE CHROMOSOMENVERHÄLTNISSE BEI DEN UNTERSUCHTEN CURCULIONIDEN.

### 1. DIE BISEXUELLEN ARTEN UND RASSEN.

Wie aus dem auf S. 426 Gesagten hervorgeht, betrifft die vorliegende Arbeit die Chromosomenverhältnisse bei insgesamt 13 bisexuellen *Otiorrhynchus*-Arten. Die Spermatogenese ist bei 11 Arten und die Oogenese bei 10 Arten untersucht worden. Die untersuchten Individuen verteilen sich auf die verschiedenen Arten wie folgt: *O. inflatus* 1 ♂; *O. geniculatus* 2 ♂♂, 1 ♀; *O. sensitivus* 3 ♂♂, 3 ♀♀, *O. armadillo* 1 ♂, 1 ♀; *O. bisulcatus* 1 ♂, 1 ♀; *O. niger* 2 ♂♂, 5 ♀♀ (dazu wurden 5 parthenogenetische ♀♀ untersucht); *O. fuscipes* 2 ♂♂; *O. morio* 2 ♂♂, 4 ♀♀; *O. austriacus* 1 ♀; *O. equestris* 2 ♂♂; *O. salicis* 2 ♂♂, 1 ♀ (dazu 3 parthenogenetische ♀♀ untersucht); *O. gemmatus* 3 ♂♂, 1 ♀ (dazu 4 parthenogenetische ♀♀ untersucht); *O. chrysops* 4 ♀♀. Dieses Material stammt in seiner Gänze aus Lunz am See.

Weil die untersuchten bisexuellen *Otiorrhynchus*-Arten alle dieselbe Chromosomenzahl haben und weil sie auch in bezug auf die Meiose einander ähneln, werden sie im folgenden gemeinsam behandelt. Die Grösse der Chromosomen variiert jedoch einigermaßen von Art zu Art.

### A. DIE SPERMATOGENESE.

Die diploide Chromosomenzahl des Männchens beträgt bei sämtlichen untersuchten Arten 22; dieselbe diploide Chromosomenzahl habe ich auch bei den von mir früher untersuchten bisexuellen Curculionidenarten festgestellt (vgl. SUOMALAINEN, 1940 a und b). Zwei von den Chromosomen sind Geschlechtschromosomen. Das Geschlechtschromosomenpaar besteht beim *Otiorrhynchus*-Männchen aus einem X- und einem sehr kleinen Y-Chromosom.

*Spermatogonienchromosomen.* — Es ist mir gelungen Spermatogonienpräparate nur von wenigen Arten zu erhalten. In Fig. 1 ist eine Metaphaseplatte bei *Otiorrhynchus fuscipes* abgebildet. Die meisten von den 20 Autosomen, vielleicht alle, haben ein nahezu medianes Zentromer. Ein Chromosom, das wahrscheinlich das X-Chromosom ist, scheint telozentrisch zu sein. Das Y ist rundlich und recht klein.

Die früheren Stadien der Meiose verlaufen nach dem gewöhnlichen Schema. Im Leptotän sieht man im Kern lange, dünne Chromosomenfäden, die unregelmässig gewunden sind und einen dichten Knäuel bilden. Im späten Leptotän und im frühen Pachytän kann man eine



Fig. 1. *Otiorrhynchus fuscipes*. Fig. 2—3 und 6—8. *O. sensitivus*. Fig. 4. *O. morio*. Fig. 5. *O. salicis*. — Fig. 1. Spermatogonienmetaphase. — Fig. 2. Diplotäntetraden beim ♂. — Fig. 3. Tetraden der frühen Diakinese beim ♂. — Fig. 4. Diakinese beim ♂. — Fig. 5—6. Profilbilder der 1. Metaphase beim ♂ (←→). — Fig. 7. Profilbild der 1. Anaphase beim ♂. — Fig. 8. Profilbild der 2. Metaphase beim ♂ (nicht alle Chromosomen sind eingezeichnet). — Fig. 1 und 5—7. KES. Fig. 2—4 und 8. KES + B—A + Feulg.

recht deutliche Bukettorientierung der Chromosomen wahrnehmen. Weil ich kein genau analysierbares Zygotänstadium gefunden habe,

lässt es sich nicht ermitteln, wie die Konjugation im einzelnen stattfindet. Im späteren Pachytän verschwindet die Bukettorientierung, und die Chromosomen, die jetzt ihrer ganzen Länge nach eng konjugiert sind, liegen nun zerstreut im Kernraum. Im Pachytän sieht man in allen Kernen einen verhältnismässig grossen Nukleolus, der deutlich dem heteropyknotischen X-Chromosom angeheftet ist. Auch das Y-Chromosom scheint mit dem Nukleolus zusammenzuhängen.

*Tetradengenese.* — Nach dem Pachytän trennen sich die konjugierten homologen Chromosomen schnell voneinander, womit das Diplotänstadium beginnt. Ein besonderes Strepsitänstadium kann bei keiner Art wahrgenommen werden. Im Diplotän und in der Diakinese gewahrt man bei sämtlichen Arten typische und deutliche Chiasmata. Sowohl deren Symmetrischwerden als deren Terminalisation finden auf allgemein bekannte Weise statt. Im Diplotän und in den Anfangsstadien der Diakinese (Fig. 2—3) ist die Kontraktion der Chromosomen noch gering, sodass dieselben lang und schlank erscheinen. Die Chiasmata sind nur in wenigen Fällen symmetrisch geworden und ihre Terminalisation befindet sich erst im Anfang. Die meisten Tetraden haben deutlich nur ein Chiasma; doch sieht man auch Tetraden mit zwei Chiasmata. Ein paarmal habe ich auch solche Tetraden gefunden, bei denen anscheinend drei Chiasmata vorkommen (vgl. Fig. 2). Die dritte als ein Chiasma erscheinende Stelle in ihnen kann natürlich auch eine durch Torsion der Partner herbeigeführte optische Überkreuzung sein.

In der späteren Diakinese (Fig. 4) ist die Kontraktion deutlich fortgeschritten, sodass die Chromosomen kürzer und dicker sind. Die Chiasmata sind im allgemeinen schon symmetrisch und oft auch vollständig terminalisiert. Die meisten Tetraden sind typische Stabtetraden; man sieht jedoch auch Kreuztetraden, sogar solche mit verhältnismässig langen Querbalken. Neben den erwähnten Tetradentypen findet man auch typische Ringtetraden mit zwei an verschiedenen Enden terminalisierten Chiasmata. Die Zahl der Ringtetraden in einer Zelle ist bei verschiedenen Arten ein wenig verschieden, wie der folgende Vergleich zwischen *O. sensitivus* und *O. morio* (gezählt an 50 Spermatozyten) zeigt:

	Zellen ohne Ringtetr.	Zellen mit 1 Ringtetr.	Zellen mit 2 Ringtetr.	Zellen mit 3 Ringtetr.	Zellen mit 4 Ringtetr.	Zellen mit 5 Ringtetr.	Zellen mit 6 Ringtetr.
<i>O. sensitivus</i> . . . .	15	18	11	6	—	—	—
<i>O. morio</i> . . . . .	2	4	12	13	12	6	1

Bei *O. sensitivus* schwankt demgemäss die Zahl der Ringtetraden in einer Zelle zwischen 0 und 3 und bei *O. morio* zwischen 0 und 6. Bei

der ersteren Art findet man in einem Kern am häufigsten also einen Ring, bei *O. morio* aber 3 solche.

*Reifungsteilungen.* — In der 1. Metaphase gewahrt man 10 Autosomentetraden und das XY-Paar (Fig. 5—6). Die Lage der Tetraden wird durch die Koorientierung der beiden homologen Zentromeren bestimmt. Die Längsachsen der Tetraden sind nämlich parallel zur Spindel eingestellt. Die Kontraktion der Chromosomen ist jetzt maximal und die Chiasmata sind mit wenigen Ausnahmen völlig terminalisiert. In den meisten Metaphaseplatten findet man auch einige Ringtetraden mit zwei terminalisierten Chiasmata. Die zusammenhängenden X- und Y-Chromosomen sind nach verschiedenen Polen gerichtet.

In der 1. Anaphase (Fig. 7) trennen sich die konjugierten Chromosomen auf gewöhnliche Weise voneinander. In beiden Platten sieht man 10 Autosomen; dazu kommt in der einen Platte noch das X-Chromosom und in der anderen das kleine Y-Chromosom. Als Folge der 1. Reifungsteilung entstehen somit zweierlei 2. Spermatocyten, nämlich sowohl solche mit dem X- als solche mit dem Y-Chromosom. In der 2. Metaphase liegen die Längsachsen der Chromosomen in der Richtung der Äquatorialebene. In Seitenansicht sind die Chromosomen oft kreuzförmig (Fig. 8), weil ihre Chromatiden nur an der Spindelansatzstelle aneinander haften. Bei der 2. Reifungsteilung teilen sich die Chromosomen äquationell.

Der Konjugationsmechanismus der X- und Y-Chromosomen wird in anderem Zusammenhang näher behandelt.

## B. DIE OOGENESE.

Die diploide Chromosomenzahl des Weibchens ist bei allen untersuchten Arten dieselbe wie beim Männchen, nämlich 22. Beim Weibchen sind die beiden Geschlechtschromosomen X-Chromosomen.

Oogonienteilungen sind in meinen Präparaten nicht vorhanden. Auch die früheren Stadien der Meiose sowie die Tetradengnese wurden in der Oogenese nicht näher untersucht.

*Reifungsteilungen.* — In der 1. Metaphase gewahrt man bei allen untersuchten bisexuellen Arten 11 Tetraden, von denen eine das konjugierte X-Chromosomenpaar ist (Fig. 9—13). Sie sind meistens sehr regelmässig zur 1. Metaphaseplatte angeordnet. Die meisten Tetraden sind Stabtetraden mit einem terminalisierten Chiasma; nicht selten findet man auch Ringtetraden mit zwei Chiasmata. Wie bei den Spermatocyten, so scheint auch bei den Oocyten die Zahl der Ring-

tetraden in einer Zelle bei verschiedenen Arten ein wenig verschieden zu sein. Eine Untersuchung der Oozyten von *O. sensitivus* und *O. morio* gab nämlich in dieser Hinsicht folgendes Resultat.

	Zellen ohne Ringtetr.	Zellen mit 1 Ringtetr.	Zellen mit 2 Ringtetr.	Zellen mit 3 Ringtetr.	Zellen mit 4 Ringtetr.	Zellen mit 5 Ringtetr.
<i>O. sensitivus</i> . . . . .	2	2	2	—	—	—
<i>O. morio</i> . . . . .	1(0?)	1(2?)	3	2	—	1

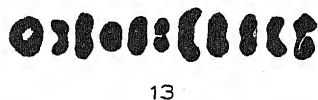
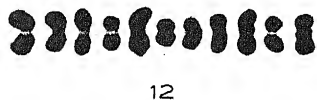


Fig. 9. *Otiorrhynchus morio*. Fig. 10. *O. niger*. Fig. 11—12. *O. salicis*. Fig. 13. *O. gemmatus*. — Fig. 9 und 12—13. Profilbilder der 1. Metaphase beim ♀ der bisexuellen Rasse (←→). — Fig. 10—11. Metaphaseplatten der 1. Reifungsteilung beim ♀ der bisexuellen Rasse. — Alles C + Feulg.

In bezug auf die Zahl der Ringtetraden scheint demgemäss kein grösserer Unterschied zwischen Spermatozyten (vgl. S. 430) und Oozyten zu bestehen. (Das Material ist allerdings nicht gross. Besonders in den Oozyten ist es nämlich nicht leicht die Zahl der Ringtetraden zu bestimmen, weil die Ringe in den Schnittpräparaten teilweise auch im Profil sichtbar sind.)

Die Eier befinden sich bei der Ablage im Metaphasestadium der 1. Teilung. Die beiden Reifungsteilungen erfolgen bald nachdem das Ei abgelegt worden ist. Sie weisen keine Besonderheiten in bezug auf ihren Ablauf auf. In der 2. Teilung teilt sich die innere Platte früher als die äussere, soweit sich die letztgenannte überhaupt teilt.

Bemerkenswert ist der Umstand, dass von drei Arten, nämlich *Otiorrhynchus niger*, *O. salicis* und *O. gemmatus*, in Lunz am See neben diploiden bisexuellen Weibchen auch triploide Weibchen auftreten, die recht wahrscheinlich parthenogenetisch sind.

## 2. DIE PARTHENOGENETISCHEN ARTEN UND RASSEN.

In der vorliegenden Untersuchung werden die Chromosomenverhältnisse bei insgesamt 10 parthenogenetischen Arten bzw. Rassen be-

handelt. Von diesen sind acht triploid, eine tetraploid und eine pentaploid. Bei sämtlichen machen die Eizellen nur eine Reifungsteilung durch, die eine Äquationsteilung ist.

In der Literatur sind mir keine Angaben über Parthenogenese bei fünf von den untersuchten Arten, nämlich *Otiorrhynchus niger*, *O. singularis*, *O. gemmatus*, *O. pupillatus* und *Barynotus moerens*, begegnet.

#### A. DIE TRIPLOIDEN ARTEN UND RASSEN.

*Otiorrhynchus niger*. — Insgesamt zehn Weibchen wurden untersucht. Sie stammen alle aus Lunz am See. Fünf von den Tieren waren diploid mit normaler Chromosomenkonjugation und Tetradenbildung (siehe Fig. 10); sie gehören demnach der bisexualen Rasse an. Fünf von den untersuchten Weibchen waren dagegen triploid mit 33 Chromosomen. Ihre Eier laufen nur eine Reifungsteilung durch und entwickeln sich darum wahrscheinlich parthenogenetisch. Bei den parthenogenetischen Weibchen kann man in der Metaphaseplatte der Reifungsteilung 33 ungleich grosse univalente Chromosomen finden (Fig. 14). Die exakte Bestimmung der Chromosomenzahl war freilich nur in einer Platte möglich. Platten mit etwas über 30 Chromosomen waren in den Präparaten mehrere enthalten. Eine genauere Analyse der Chromosomentypen ist bei keiner untersuchten Art möglich.

*Otiorrhynchus scaber*. — Diese Art ist in verschiedenen Gegenden Südfinnlands tetraploid (SUOMALAINEN, 1940 a, S. 58; 1940 b, S. 88—91). Bei den finnischen Exemplaren konnten nämlich in den Metaphaseplatten der Reifungsteilung in den meisten Fällen 44 Chromosomen gezählt werden; man findet jedoch auch Platten mit 42 oder 43 Chromosomen.

Die Art war häufig auf einer ganz beschränkten Fläche in der Nähe der Biologischen Station in Lunz. Sie war dort parthenogenetisch; die gesammelten 130 Exemplare waren alle Weibchen. *Otiorrhynchus scaber* erwies sich in Lunz am See als triploid (vgl. SUOMALAINEN, 1944; 1945, S. 187—188). Insgesamt zehn Weibchen wurden zytologisch untersucht und sie waren alle triploid. In sämtlichen acht Eiern, die eine exakte Bestimmung der Chromosomenzahl erlaubten, wurde diese als 33 gefunden (Fig. 15). Ausserdem wurden in mehreren Metaphaseplatten je etwas über 30 Chromosomen festgestellt. Die triploide Chromosomenzahl wurde auch in Follikelzellen des Ovars konstatiert.

In einem von den untersuchten Ovarien waren in den Prophasekernen weniger als 33 Chromosomenelemente sichtbar. Freilich nur

zwei späte Prophasen wurden gefunden; die eine enthielt 21—22 Chromosomenelemente, die andere nur ungefähr 16. Mehrere von diesen Chromosomenelementen waren bedeutend grösser als die univalenten

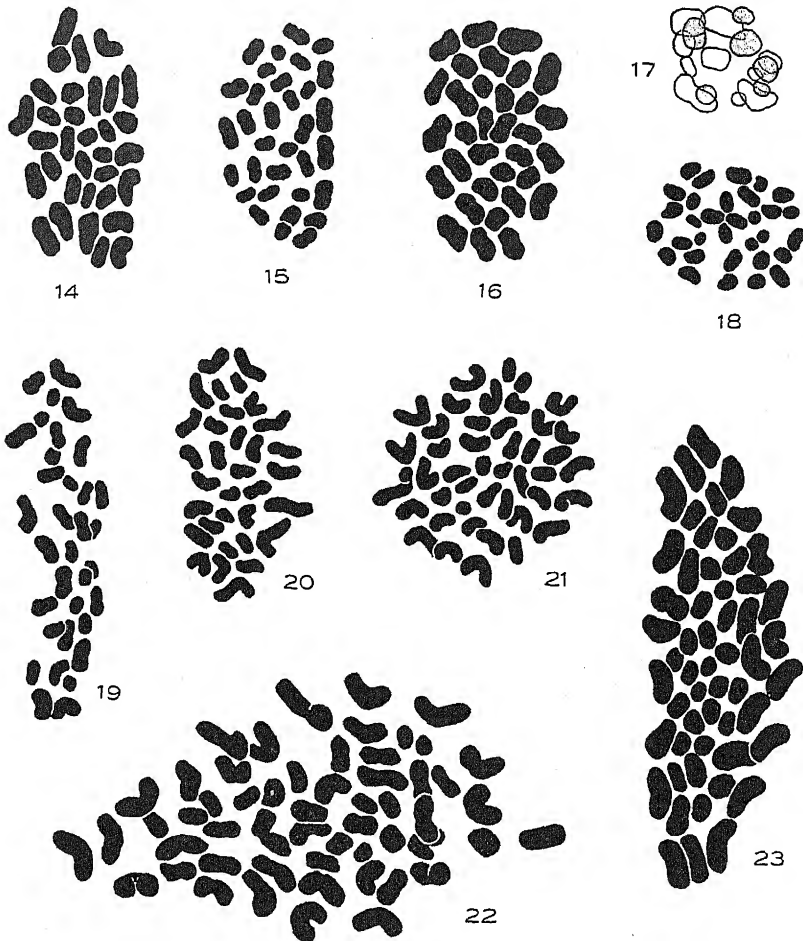


Fig. 14. *Otiorrhynchus niger*. 33 Chromosomen. — Fig. 15. *O. scaber*. 33 Chromosomen. — Fig. 16. *O. singularis*. 33 Chromosomen. — Fig. 17—18. *O. salicis*. 32 Chromosomen. — Fig. 19. *O. sulcatus*. 34 Chromosomen. — Fig. 20. *O. gemmatus*. 33 Chromosomen. — Fig. 21. *O. pupillatus*. 44 Chromosomen. — Fig. 22—23. *Barynotus moerens*. 55 Chromosomen. — Fig. 17. Prophase der Reifungsteilung des parthenogenetischen ♀. Fig. 14—16 und 18—23. Metaphasen der Reifungsteilung des parthenogenetischen ♀. — Alles C + Feulg.

Metaphasechromosomen. Es ist offenbar, dass jene wenigstens zwei Chromosomen enthalten. Diese Frage wird später (S. 435—436) näher diskutiert.

*Otiorrhynchus singularis*. — Von dieser Art wurden sechs Weibchen zytologisch untersucht. Eines von diesen stammt aus Südfinnland, aus Helsinki; die anderen fünf wurden in Mitteldeutschland, in Berlin-Buch gesammelt. Insgesamt wurden in Berlin-Buch etwa 20 *Otiorrhynchus singularis*-Exemplare erbeutet, und sie alle waren Weibchen. Sämtliche untersuchten Tiere waren triploid und zytologisch gleich. In der Metaphaseplatte der Reifungsteilung sind 33 univalente, oft ein wenig semmelförmige Chromosomen zu finden (Fig. 16). Die exakte Bestimmung der Chromosomenzahl war freilich nur in drei Platten möglich; ausserdem wurden in mehreren Platten ungefähr 33 Chromosomen konstatiert. Die meisten Chromosomen zeigen in ihrer Mitte eine mehr oder weniger deutliche Einschnürung und scheinen daher ein nahezu medianes Zentromer zu haben. (Die nahezu mediane Lage des Zentromers ist auch bei einigen anderen Arten [vgl. die Figuren] bei der Reifungsteilung in den meisten Chromosomen feststellbar.)

*Otiorrhynchus salicis*. — Vier Weibchen, alle aus Lunz am See, wurden untersucht. Eines von denselben war diploid mit normaler Chromosomenkonjugation und Tetradenbildung (siehe Fig. 11—12). Dieses Weibchen gehört der bisexuellen Rasse an. Drei von den untersuchten Weibchen waren dagegen triploid. Ihre Eier machen nur eine Reifungsteilung durch und entwickeln sich wahrscheinlich parthenogenetisch. Bei den parthenogenetischen Weibchen war die exakte Bestimmung der Chromosomenzahl nur in einer Metaphaseplatte der Reifungsteilung möglich; sie beträgt 32 (Fig. 18). Platten mit etwas über 30 univalenten Chromosomen wurden mehrere gefunden. Bei einem von den untersuchten Weibchen waren die Chromosomen in den Eiern nicht zu einer regelmässigen Metaphaseplatte angeordnet, sondern ganz unregelmässig gruppiert. Die triploide Chromosomenzahl war jedoch auch hier feststellbar.

Bemerkenswert ist der Umstand, dass in den Eiern eines parthenogenetischen *O. salicis*-Weibchens — ganz wie es bei einem *O. scaber*-Weibchen (vgl. S. 434) der Fall war — während der späten Prophase bedeutend weniger als 32—33 Chromosomenelemente sichtbar waren (Fig. 17). Insgesamt wurden über zehn solche Prophasen gefunden. Weil die Chromosomenelemente in der späten Prophase in einer dichten Gruppe stehen, lässt sich ihre Zahl meistens nicht exakt ermitteln. In den fraglichen Prophasen schwankt die Zahl der Chromosomenelemente zwischen etwa 7 und ungefähr 20; meistens beträgt sie etwa 20. Die in Fig. 17 abgebildete Zelle z. B. hat 16 Chromosomenelemente. Die Grössenunterschiede derselben sind viel beträchtlicher als diejenigen der

univalenten Chromosomen in den triploiden Metaphaseplatten. Es ist offenbar, dass die grossen Chromosomenelemente dieser Prophasekerne zwei oder mehrere Chromosomen enthalten. Es ist schwierig mit Sicherheit zu entscheiden, ob die sich zusammenschliessenden Chromosomen homolog sind und an den Chiasmata aneinander haften — also Bivalente oder Trivalente bilden — oder ob zwei oder mehrere Chromosomen sich ohne Chiasmabildung vorübergehend aneinander anschliessen. Der Umstand, dass in entsprechenden Metaphaseplatten nie Bivalente oder Trivalente auftreten und dass in einem Prophasekern die Zahl der Chromosomenelemente sogar weniger als 11 (etwa 7) betrug, spricht für die letztgenannte Alternative.

*Otiorrhynchus sulcatus*. — Diese in Finnland sonst sehr seltene Art ist häufig auf einer beschränkten Fläche im Botanischen Garten der Universität Helsinki. Sie ist dort parthenogenetisch, denn unter den ungefähr 150 untersuchten Exemplaren wurde kein einziges Männchen gefunden. Insgesamt zehn Weibchen wurden zytologisch untersucht, und sie waren alle triploid. In der Metaphaseplatte der Reifungsteilung kann man bei dieser Art 34 univalente Chromosomen sehen (Fig. 19). Alle fünf Eier, die eine exakte Bestimmung der Chromosomenzahl erlaubten, zeigten in der Metaphaseplatte 34 Chromosomen. Platten mit etwas über 30 Chromosomen waren in den Präparaten reichlich enthalten.

*Otiorrhynchus gemmatus*. — Fünf Weibchen, alle aus Lunz am See, wurden untersucht. Eines von denselben war diploid mit normaler Chromosomenkonjugation und Tetradenbildung (siehe Fig. 13); es gehört der bisexuellen Rasse an. Vier von den untersuchten Weibchen waren dagegen triploid mit 33 Chromosomen. Weil ihre Eier nur eine Reifungsteilung durchlaufen, entwickeln sie sich offenbar parthenogenetisch. Diese beiden rassenspezifischen Eiertypen wurden auch unter abgelegten Eiern konstatiert. Bei den parthenogenetischen Weibchen kann man in der Metaphaseplatte der Reifungsteilung 33 ungleich grosse univalente Chromosomen finden (Fig. 20). Die exakte Bestimmung der Chromosomenzahl war in fünf Platten möglich. In mehreren Platten wurden etwas über 30 Chromosomen konstatiert.

*Sciaphilus asperatus*. — Diese Art ist in verschiedenen Gegenden Südfinnlands triploid mit 33 Chromosomen (SUOMALAINEN, 1940 a, S. 57; 1940 b, S. 82—84).

Zwei Weibchen aus Berlin-Buch wurden zytologisch untersucht. Auch sie waren triploid, es wurden nämlich in den Eiern in der Metaphaseplatte der Reifungsteilung 33 Chromosomen festgestellt.

*Strophosomus melanogrammus*. — Diese Art ist auf Åland im südwestlichen Finnland triploid. Die Chromosomenzahl variiert in den Oozyten von 31 bis 35. Als häufigste Chromosomenzahl ergibt sich 34 (SUOMALAINEN, 1940 a, S. 56; 1940 b, S. 78—80).

Auch von dieser Art wurden zwei in Berlin-Buch gesammelte Weibchen untersucht. Beide waren triploid. Von den fünf Eiern, die eine exakte Bestimmung der Chromosomenzahl gestatteten, wurden bei vier in der Metaphase der Reifungsteilung 34 Chromosomen konstatiert; das fünfte hatte 33 Chromosomen. Wie bei den finnischen Exemplaren, gewahrt man auch hier ein im Vergleich zu den übrigen deutlich kleineres Chromosom.

#### B. DIE TETRAPLOIDEN ARTEN UND RASSEN.

Von den zehn parthenogenetischen Rüsselkäferarten, deren Chromosomenverhältnisse in dieser Untersuchung näher behandelt werden, erwies sich nur eine als tetraploid.

*Otiorrhynchus pupillatus*. — Die Art ist in Lunz am See parthenogenetisch; die gesammelten 26 Exemplare waren alle Weibchen. Vier von diesen wurden zytologisch untersucht. Sie waren alle tetraploid. Wegen der grossen Zahl der Chromosomen war ihre exakte Zählung nur in zwei Metaphaseplatten der Reifungsteilung möglich. Beide enthielten 44 univalente Chromosomen (Fig. 21). Ausserdem wurden in mehreren Metaphaseplatten ungefähr 44 Chromosomen festgestellt. Die grösseren Chromosomen sind langgestreckt, in ihrer Mitte deutlich gebogen.

#### C. DIE PENTAPLOIDEN ARTEN UND RASSEN.

Eine von den untersuchten Arten erwies sich als pentaploid.

*Barynotus moerens* (= *elevatus*). — Zwei Weibchen aus Lunz am See wurden untersucht. Sie waren beide pentaploid mit 55 Chromosomen. Ungeachtet der recht grossen Chromosomenzahl gelang es mir in drei Metaphaseplatten der Reifungsteilung exakt die genannte Zahl von Chromosomen zu konstatieren (Fig. 22—23). Ausserdem waren in sechs Metaphaseplatten ungefähr 55 Chromosomen feststellbar. Dazu kommen noch einige Platten, die etwas über 50 Chromosomen zeigen. Die Pentaploidie wurde also bei dieser Art ganz einwandfrei festgestellt. Ganz wie bei der anderen, früher untersuchten *Barynotus*-Art, *B. obscurus* (vgl. SUOMALAINEN, 1940 a and b), sind die Chromosomen von *B. moerens* recht gross und zeigen oft ein deutliches, nahezu medianes Zentromer.

Wenngleich die Art in Finnland recht selten ist, gelang es mir nach eifrigem Suchen zwei *Barynotus moerens*-Weibchen (die Bestimmung dieser Exemplare ist von Herrn Dozent Dr. ERNST PALMÉN bestätigt worden) aus dem Botanischen Garten der Universität Helsinki zur Untersuchung zu bekommen. Leider war es nicht möglich, ihre Chromosomenzahl genau festzustellen. Sie scheinen jedoch viel weniger Chromosomen zu haben als die pentaploiden Exemplare aus Lunz. Die finnischen *Barynotus moerens*-Weibchen repräsentieren somit eine niedrigere Polyploidiestufe (vielleicht Triploidie?) als die untersuchten österreichischen Exemplare. Ich hoffe neues Material zu erhalten, um diese interessanten Chromosomenverhältnisse genauer untersuchen zu können.

#### D. DIE ORIENTIERUNG DER CHROMOSOMEN IN DER METAPHASEPLATTE BEI DEN PARTHENOGENETISCHEN ARTEN.

In den Eiern mancher von mir (SUOMALAINEN, 1940 a und b) früher untersuchten polyploiden Rüsselkäfer lässt sich ziemlich allgemein eine eigenartige Gonomerie wahrnehmen. Die Chromosomen können sich in der Metaphase der Reifungsteilung zu zwei oder sogar drei getrennte Platten anordnen. Drei Platten findet man jedoch nur bei tetraploiden Arten. Jede der Platten enthält im allgemeinen ein oder mehrere volle Genome. Diese getrennten Platten können weit voneinander entfernt liegen.

Auch bei den in der vorliegenden Untersuchung beschriebenen polyploiden Curculionidenarten wurde eine ähnliche Genomsonderung festgestellt. Bei allen sechs triploiden *Otiorrhynchus*-Arten wurden neben Eiern mit einer triploiden Metaphaseplatte auch solche Eier gefunden, die eine diploide und eine haploide Platte aufweisen. Die Zahl der letztgenannten Eier war jedoch im allgemeinen ziemlich gering, sie schwankte nämlich lediglich zwischen 2,5 % (*O. singularis*) und 12,5 % (*O. salicis*). (Dasjenige *O. salicis*-Weibchen, dessen Eier eine ganz unregelmässige Gruppierung der Metaphasechromosomen zeigen [vgl. S. 435], ist hierbei nicht berücksichtigt.) Nur bei *O. niger* sind ziemlich viele, nämlich 25 % der Eier mit Teilplatten zu finden. Beim tetraploiden *O. pupillatus* befanden sich unter 22 Eiern zwei mit einer triploiden und einer haploiden Platte und eines mit zwei diploiden Platten (das Weibchen mit »degenerierenden« Eiern [vgl. unten] scheidet hierbei aus) und beim pentaploiden *Barynotus moerens* befand sich unter 32 Eiern eines mit einer triploiden und einer diploiden Platte.

Eines von den untersuchten *Otiorrhynchus pupillatus*-Weibchen zeigt in seinem Ovarium mehrere Eier, deren Plasma Anzeichen einer beginnenden Degeneration aufweist. In diesen Eiern kann man 2—3 weit voneinander entfernt liegende degenerierende Chromosomengruppen wahrnehmen, die verhältnismässig wenig (eine haploide oder diploide Zahl?) Chromosomen enthalten. Es ist möglich, dass wir es hier mit solchen Fällen zu tun haben, in welchen die Teilplatten so weit voneinander entfernt liegen, dass eine normale Entwicklung des Eies nicht mehr möglich ist.

### III. BESPRECHUNG DER ERGEBNISSE.

#### 1. DIE VERSCHIEDENEN RASSEN EIN UND DERSELBEN ART MITEINANDER VERGlichen.

Oben (S. 426) ist bereits davon die Rede gewesen, dass man bei manchen Rüsselkäferarten sowohl eine bisexuelle als eine parthenogenetische Rasse kennt. Einige andere Arten vermehren sich nur parthenogenetisch; bei ihnen ist keine bisexuelle Rasse festgestellt worden. Doch sind in vielen solchen Fällen ganz nahe verwandte bisexuelle Arten bekannt, die möglicherweise als die bisexuellen Gegenstücke der betr. Arten gelten könnten, obwohl sie als eigene Arten bezeichnet worden sind. Es hat mir bisher an Gelegenheit gefehlt, die Chromosomenverhältnisse solcher einander entsprechenden fortpflanzungsbiologisch verschiedenen Curculionidenrassen zu untersuchen. In vorliegender Arbeit sind jedoch sowohl die bisexuelle als die parthenogenetische Rasse dreier *Otiorrhynchus*-Arten, nämlich *O. niger*, *O. salicis* und *O. gemmatus*, zytologisch untersucht worden. Bei allen diesen Arten ist die bisexuelle Rasse diploid, und ihre Eier laufen zwei Reifungsteilungen durch. Die parthenogenetische Rasse ist dagegen polyploid (triploid); die Eier dieser Rasse machen nur eine Reifungsteilung, die Äquationsteilung, durch. In allen diesen Fällen ist die parthenogenetische Vermehrung also mit Polyploidie verbunden. Bemerkenswert ist auch, dass bei den Rüsselkäfern, wenn die Vermehrung parthenogenetisch wird, die Tetradenbildung wie auch die Chromosomenreduktion ausfallen.

Auch zytologisch verschiedene parthenogenetische Rassen wurden bei einigen Curculionidenarten konstatiert. So wurden bei *Otiorrhynchus scaber* zwei verschiedene parthenogenetische Rassen, nämlich eine triploide und eine tetraploide, festgestellt. Entsprechenden Verhältnissen begegnen wir bei *Barynotus moerens*, der neben einer penta-

ploiden parthenogenetischen Rasse eine andere parthenogenetische Rasse hat, die eine niedrigere Polyploidiestufe repräsentiert. Bei *Otiorrhynchus scaber* tritt in den Ostalpen in einigen ganz beschränkten Gebieten (Näheres auf S. 444) noch eine bisexuelle Rasse auf, die mit recht grosser Wahrscheinlichkeit diploid ist. *Otiorrhynchus scaber* ist somit eine Art, von der es wenigstens drei verschiedene Rassen gibt, nämlich eine diploide bisexuelle, eine triploide parthenogenetische und eine tetraploide parthenogenetische.

Bei manchen polyploiden Tieren tritt als Folgeerscheinung der Polyploidie Gigantismus auf; bei anderen wiederum ist solches nicht nachweisbar (Näheres z. B. bei FANKHAUSER, 1945, S. 48—50). HOFF-

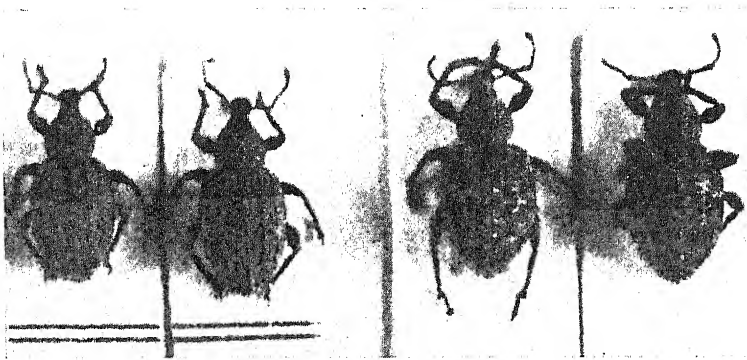


Fig. 24. Triploide *Otiorrhynchus scaber*-Individuen aus den Österreichischen Kalkalpen (Lunz am See) (links) und tetraploide finnische Individuen derselben Art (rechts). 4,5 $\times$ . — Der Farbenunterschied der Käfer beruht auf der die ersteren bedeckenden feinen staubartigen Schicht.

MANN (1932) hat bezüglich zweier Arten der Curculionidengattung *Trachyploeus*, *Tr. alternans* und *Tr. spinimanus*, festgestellt, dass bei ihnen die bisexuelle Rasse um die Hälfte kleiner als die parthenogenetische (und wahrscheinlich polyploide; vgl. VANDEL, 1932) ist. Die verschiedenen Rassen von *Otiorrhynchus niger*, *O. salicis* und *O. gemmatus* wurden leider erst bei der zytologischen Untersuchung festgestellt, als ein Vergleich ihrer Körpergrösse nicht mehr möglich war. Die beiden zytologisch verschiedenen parthenogenetischen Rassen von *Otiorrhynchus scaber* liessen sich dagegen in betreff ihrer Körpergrösse miteinander vergleichen. Wie aus Fig. 24 hervorgeht, ist die triploide Rasse aus Lunz am See deutlich etwas kleiner als die tetraploide aus Finnland; der Unterschied ist allerdings nicht gross.

Die Eier der verschiedenen Rassen zeigen in bezug auf ihre Grösse

— soweit man dies aus den Schnittpräparaten beurteilen kann — keine grösseren Unterschiede. Der Querdurchmesser der Eier der diploiden bisexuellen Rasse von *Otiorrhynchus niger* ist 0,55 mm und derjenige der Eier der triploiden parthenogenetischen Rasse 0,57 mm. Bei *O. salicis* sind die entsprechenden Werte 0,43 mm und 0,43 mm und bei *O. gemmatus* 0,61 mm und 0,65 mm (alles Mittelwerte von 10 reifen Eiern). Entsprechend liegt die Sache bei *O. scaber*; der Querdurchmesser der Eier der triploiden parthenogenetischen Rasse ist nämlich 0,37 mm und derjenige der tetraploiden Rasse ebenfalls 0,37 mm (Mittelwerte von 20 reifen Eiern). Die Grössenverhältnisse der Eier verschiedener Curculionidenrassen ähneln somit denjenigen bei *Solenobia*, deren verschiedene Rassen auch gleich grosse Eier haben (SEILER, 1936, S. 150).

## 2. DIE VERBREITUNG DER VERSCHIEDENEN RASSEN EIN UND DERSELBEN ART.

Wenn von zwei naheverwandten Arten oder von zwei Rassen ein und derselben Art die eine bisexuell und die andere thelytok parthenogenetisch ist, besitzen die beiden Arten bzw. Rassen gewöhnlich wenigstens zum Teil ihre eigenen Verbreitungsgebiete, und zwar oft so, dass die parthenogenetische Form die nördlichere ist (siehe z. B. VANDEL, 1928, S. 244 ff.; 1931, S. 174—200). Wegen der verschiedenen Verbreitung der parthenogenetischen Form werden derartige Fälle als geographische Parthenogenese bezeichnet (VANDEL, op. c.).

Die direkte Ursache zu der geographischen Parthenogenese ist wenigstens in den meist typischen Fällen jedoch nicht die Parthenogenese selbst, sondern die den parthenogenetischen Formen eigene Polyploidie. Wo die bisexuelle und die parthenogenetische Rasse eine deutlich verschiedene Verbreitung aufweisen, hat sich die letzterwähnte meistens als polyploid erwiesen. Besonders deutliche Beispiele von solchen Fällen bieten uns u. a. die parthenogenetischen Rassen von *Artemia salina* (ARTOM, 1911, 1931; GROSS, 1932; BARIGOZZI, 1934, 1935), *Trichoniscus elisabethae* (VANDEL, 1926, 1928, 1931, 1934), *Sagapedo* (MATTHEY, 1941, 1946; GOLDSCHMIDT, 1946), manchen Phasmiden (über diesbezügliche Literatur näher bei WHITE, 1945, S. 295), und *Solenobia triquetrella* (SEILER, 1923, 1927, 1939, 1942, 1943). Obwohl bei allen diesen Tieren die Polyploidie die eigentliche Ursache der geographischen Parthenogenese ist, ist anderseits zu bemerken, dass ihre Polyploidie gerade durch die Parthenogenese ermöglicht ist (Näheres bei SUOMALAINEN, 1940 b, S. 122 ff.).

Die verschiedene Verbreitung der diploiden bisexuellen und der ihr entsprechenden polyploiden parthenogenetischen Rasse ist bei manchen Curculionidenarten recht deutlich feststellbar. Ein ausgezeichnetes Beispiel dafür bietet *Otiorrhynchus dubius*. Diese Art hat eine typische sog. boreoalpine Verbreitung (SZÉKESSY, 1937, S. 582; HOLDHAUS und LINDROTH, 1939, S. 215—218). In ihrem borealen Verbreitungsgebiet (Grönland, Island, Färöer, Grossbritannien, Irland, Dänemark, Fennoskandien und Nordrussland) ist sie ausschliesslich parthenogenetisch und wenigstens in einem Teil des Gebietes polyploid (in Finnland hat sich nämlich die Art als tetraploid erwiesen [SUOMALAINEN, 1940 a, S. 57; 1940 b, S. 85—88]). In seinem alpinen Verbreitungsgebiet dagegen (in manchen mitteleuropäischen Gebirgen) ist *O. dubius*, soweit vorläufig bekannt, ausschliesslich bisexuell und somit recht wahrscheinlich diploid. (Siehe hierzu die Karte bei SUOMALAINEN, 1945, S. 189.)

Auch eine andere *Otiorrhynchus*-Art, *O. salicis*, eignet sich gut als Beispiel. Nach JAHN (1941, S. 368) pflanzt sich diese Art im grössten Teil ihres Verbreitungsgebietes, so in Nordeuropa, der Schweiz, in den Sudeten, Karpathen und auf der Balkanhalbinsel, parthenogenetisch fort; für die Alpen ist jedoch das Vorkommen der bisexuellen Rasse angegeben. JAHN erwähnt indessen zwei Fundorte (Königsstuhlgebiet, Goldeckgebiet) in den Ostalpen, wo die parthenogenetische Rasse festgestellt worden ist. Wie früher (S. 435) erwähnt wurde, kommen die beiden Rassen in Lunz am See nebeneinander vor; die bisexuelle Rasse erwies sich als diploid und die parthenogenetische als triploid.

Es ist klar, dass die bisexuelle Rasse bei allen diesen Arten als ursprünglich zu gelten hat. Diese Curculionidenarten bieten ein gutes Beispiel dafür, wie eine später entstandene polyploide parthenogenetische Form sich in ganz neue Gebiete auszubreiten vermocht hat. Sehr aufschlussreich in dieser Beziehung sind alle diejenigen *Otiorrhynchus*-Arten, von denen in den Alpen sowohl eine bisexuelle als auch eine parthenogenetische Rasse anzutreffen sind. JAHN (1941) erwähnt 8 solche Arten, nämlich:

<i>O. foraminosus</i>	<i>O. azaleae</i>	<i>O. auricomus</i>
<i>O. chaldeus</i>	<i>O. alpicola</i>	<i>O. chrysocomus</i>
<i>O. scaber</i>	<i>O. salicis</i>	

Zu diesen sind noch wenigstens zwei Arten hinzuzufügen, nämlich *O. niger* und *O. gemmatus*, die beide in Lunz am See sowohl eine bisexuelle als eine parthenogenetische Rasse aufweisen. Die Verbreitung der beiden verschiedenen Rassen der zwei letztgenannten Arten ist jedoch noch

nicht näher bekannt. Dagegen ist die Verbreitung aller 8 von JAHN erwähnten Arten besonders in den Ostalpen dank Professor Dr. K. HOLDHAUS sehr gut bekannt.

Aus der Karte von JAHN (op. c.; vgl. auch SUOMALAINEN, 1945, S. 190) geht deutlich hervor, dass die bisexuellen Rassen der betreffenden *Otiorrhynchus*-Arten diejenigen Alpengebiete bewohnen, die während der letzten Vereisung (der Würm-Eiszeit) eisfrei gewesen sind (»Massifs de refuge«). Eine Ausnahme macht nur *O. salicis*, dessen bisexuelle Rasse heute auch in dem damals eisbedeckten Gebiet auftritt. Die parthenogenetischen Rassen aller Arten kommen wiederum fast ausnahmslos vor in Gebieten, die während der Würm-Eiszeit von Gletschern bedeckt waren. Nach HOLDHAUS (1929, S. 979) gab es in den östlichen und südlichen Teilen der Alpen während der letzten Vereisung ausgedehnte eisfreie Gebiete, in deren tiefen Lagen auch der Wald zu wachsen vermochte. Ganz wie heute gab es an den Ost- und Südrändern der Alpen auch damals infolge der günstigen Exposition besonders in geschützten Tälern warme und trockne (xerothermische) Lokalitäten, auf welchen sich die sogar relativ wärmefordernden Vertreter der Alpenfauna die Eiszeit hindurch erhalten konnten. Die Fauna dieser Gebiete ist auch heute noch weit artenreicher und vielgestaltiger als die der ehemals eisbedeckten, zentralen und nördlichen Teile der Alpen. Nach dem Rückzug der Gletscher sind die bisexuellen Rassen der in Frage stehenden *Otiorrhynchus*-Arten in diesen Gegenden zurückgeblieben. Nur die parthenogenetischen Rassen haben sich in die während der Eiszeit vergletscherten, klimatisch ungünstigeren Gebiete auszubreiten vermocht. Ganz entsprechende Verhältnisse hat SEILER (1943) bei der Psychide *Solenobia triquetrella* in der Schweiz festgestellt.

JAHN (1941, S. 370) erklärt die verschiedene Verbreitung der bisexuellen und der parthenogenetischen Rassen so, dass »die Männchen ungünstigen Umweltverhältnissen leichter unterliegen als die Weibchen, die eine grössere ökologische Valenz besitzen dürften. Damit darf das Auftreten weiter verbreiteter Arten in parthenogenetischen Formen in den hohen Lagen der Hochgebirge und des hohen Nordens erklärt werden«. Nach ihr (l. c.) ist es auch ohne weiteres verständlich, dass »eine Rasse, die sich parthenogenetisch fortpflanzen kann, eine viel grössere Ausbreitungsfähigkeit besitzt als jene Formen, bei welchen zur Fortpflanzung Männchen benötigt werden«.

Die verschiedene Verbreitung der beiden Rassen beruht m. E. jedoch nicht darauf, dass es die Parthenogenese an sich unmittelbar ermög-

lichte, ungünstigere klimatische Verhältnisse besser zu ertragen. Die Ursache ist eben in der den parthenogenetischen Formen eigenen Polyploidie zu suchen, die allerdings, wie auf S. 441 schon erwähnt wurde, gerade durch die Parthenogenese ermöglicht wird. Es ist nämlich sehr wahrscheinlich, dass die betreffenden parthenogenetischen Rassen auch der genannten Alpen-*Otiorrhynchus*-Arten polyploid sind. Bei *O. scaber* und *O. salicis* habe ich das ja bereits festgestellt, wie oben angeführt. Die Polyploidie verändert die Reaktionsnorm und das Lebensoptimum der parthenogenetischen Rasse, und zwar oft gerade so, dass dieselbe in neue, klimatisch ungünstigere Gegenden übersiedelt. Wie wir uns aber erinnern, wurden in Lunz am See bei drei *Otiorrhynchus*-Arten sowohl eine diploide bisexuelle als eine triploide parthenogenetische Rasse gefunden. Dies kann (wenigstens in bezug auf *O. salicis*) davon herrühren, dass die verschiedenen Rassen natürlich in ihrem gemeinsamen Grenzgebiet nebeneinander vorkommen können. Auch wenn die polyploide parthenogenetische Rasse jung ist und noch nicht genug Zeit gehabt hat, in neue Gegenden überzusiedeln, können die beiden Rassen an ein und demselben Ort gemeinsam leben. Ob die polyploiden Rassen der betreffenden Curculionidenarten auch in Lunz am See den diploiden bisexuellen gegenüber in bezug auf ihre Standortsansprüche verschieden sind, z. B. in höheren Lagen leben, kann ich nicht entscheiden, weil diese verschiedenen Rassen leider erst bei der zytologischen Untersuchung festgestellt wurden.

Bemerkenswert ist, dass die Verbreitungsverhältnisse der polyploiden parthenogenetischen Tiere sehr an diejenigen mancher polyploiden Pflanzen erinnern; über diese Gegenseitigkeit sei des näheren auf die Anführungen von SUOMALAINEN (1940 b, S. 115—116) und VANDEL (1940, S. 95—97) verwiesen. Hier möge nur hervorgehoben werden, dass die Verbreitung der verschiedenen Formen von *Biscutella laevigata* in den Alpen (MANTON, 1934) in hohem Grade derjenigen der *Otiorrhynchus*-Rassen entspricht. Ganz ähnliche Verhältnisse liegen z. B. auch nach ANDERSON, WOODSON und SAX bei verschiedenen amerikanischen *Tradescantia*-Arten vor (DOBZHANSKY, 1941, S. 228).

Oben ist bereits davon die Rede gewesen, dass es von *Otiorrhynchus scaber* wenigstens drei verschiedene Rassen gibt, nämlich eine diploide bisexuelle, eine triploide parthenogenetische und eine tetraploide parthenogenetische. Es erhebt sich die Frage, wie weit diese Rassen verbreitet sind. Die bisexuelle Rasse tritt nur in einigen ganz beschränkten Gebieten in den Ostalpen (Umgebung von Graz, Wechselgebiet, Koralpen und Bürgeralm; vgl. JAHN, 1941, S. 367) auf. In den Österreichi-

schen Kalkalpen (Lunz am See) ist die Art triploid und in Finnland tetraploid.

Wie weit diese verschiedenen parthenogenetischen Rassen verbreitet sind, kann in gewissem Masse aus der Grösse der in verschiedenen Gegenden eingebrachten Sammlungsexemplare geschlossen werden, die triploiden Exemplare sind nämlich ein wenig kleiner als die tetraploiden (vgl. S. 440); irgendwelche sicheren Folgerungen können hieraus jedoch nicht gezogen werden. Einzelne Individuen beweisen in dieser Hinsicht nichts, es müssen mehrere aus demselben Ort vorliegen. Ich habe aus dem Riesengebirge und den Beskiden eine ganze Anzahl von *O. scaber*-Individuen gesehen; sie scheinen in ihrer Grösse mehr an die lunzischen als an die finnischen Individuen zu erinnern. Dies weist darauf hin, dass die triploide Rasse wenigstens in den mitteleuropäischen Gebirgsgegenden, vielleicht auch anderswo in Mitteleuropa die vorherrschende Form von *O. scaber* ist. Es ist möglich, dass die Art in Mitteleuropa triploid und in Nordeuropa tetraploid ist.

Eine endgültige Entscheidung dieser Frage sowie mancher anderen interessanten, auf die Parthenogenese und Polyploidie der Rüsselkäfer bezüglichen Probleme ist erst möglich, wenn wir viel mehr als heute von den Chromosomenverhältnissen dieser Tiere in verschiedenen Gegenden wissen.

### 3. DIE ENTSTEHUNG DER VERSCHIEDENEN RASSEN BEI DEN CURCULIONIDEN.

Obwohl die Parthenogenese und die Polyploidie bei den Tieren sehr oft in Verbindung miteinander auftreten, können wir jedoch nicht ohne weiteres voraussetzen, dass sie gleichzeitig entstehen. Das zwischen ihnen bestehende Abhängigkeitsverhältnis kann auch davon herrühren, dass die Parthenogenese bei Tieren die Polyploidie ermöglicht. Suchen wir nach den Ursachen der Polyploidie und Parthenogenese bei den Tieren, so müssen wir anderseits stets die oft geäusserte Vermutung in Betracht ziehen, dass diese bei den verschiedenen Formen in verschiedener Weise entstehen können. VANDEL (1926, 1928, 1931) z. B. nimmt an, dass die Parthenogenese und die Polyploidie (Triploidie) bei *Trichoniscus elisabethae* am wahrscheinlichsten gleichzeitig als Folge der Befruchtung eines abnormen diploiden Eies entstanden sind. Nach ihm bewirkt die auf diese Weise zustandgekommene Triploidie ein Unterbleiben der Konjugation und der Reduktion im Ei, und es entsteht ein Vorkern, der keine Befruchtungsneigung zeigt. Infolgedessen entwik-

kelt sich das Ei parthenogenetisch. Bei *Artemia salina* (ARTOM, 1911, 1931; GROSS, 1932, 1935) und *Solenobia triquetrella* (SEILER, 1923, 1927, 1942, 1943) ist die Parthenogenese jedoch sicher früher als die Polyploidie entstanden. Von beiden diesen Tieren kennt man eine diploide bisexuelle Rasse, aus welcher die polyploiden parthenogenetischen Rassen durch automiktische Vorgänge entstanden sind. Aus Obigem geht hervor, dass die polyploiden parthenogenetischen Rassen der oben-erwähnten Tiere sowie die übrigen früher untersuchten entsprechenden Tierformen als autopolyploid festgestellt oder vorausgesetzt worden sind.

Mit den Rüsselkäfern sind bisher keine eigentlichen Versuche zur Beleuchtung der Entstehung der verschiedenen Rassen angestellt worden. Ihre Züchtung ist nämlich recht schwierig, weil die meisten Vertreter der Unterfamilien Otiorrhynchinae und Brachyderinae als Larven rhizophag unter der Erdoberfläche leben. Was ich im folgenden vorbringe, trägt darum lediglich den Charakter von Hypothesen.

Versuchen wir es nun, die Entstehung der verschiedenen parthenogenetischen Curculionidenrassen zu erklären, so kommen zunächst zwei prinzipiell verschiedene Alternativen in Frage.

Die diploiden bisexuellen Rassen sind bei den Rüsselkäfern, wie auch bei den anderen parthenogenetischen Tieren, sicher ursprünglich; die parthenogenetischen Rassen sind aus ihnen entstanden. Es ist möglich, dass bei den Curculioniden ähnlich wie z. B. bei *Solenobia triquetrella* (SEILER, op. c.) aus der bisexuellen Rasse zuerst eine diploide parthenogenetische hervorgeht, d. h. zunächst eine Veränderung der Vermehrungsart eintritt, ohne dass sich die Chromosomengarnitur veränderte. Dazu könnte jemand bemerken, man kenne bisher nur eine diploide parthenogenetische Curculionidenart (*Polydrosus mollis*), während polyploide parthenogenetische Formen von insgesamt 15 Arten bekannt sind. Die Seltenheit der diploiden parthenogenetischen Rassen kann möglicherweise davon herrühren, dass sie wie die diploiden bisexuellen Rassen mancher Arten (z. B. *Otiorrhynchus scaber*) innerhalb ganz beschränkter Gebiete auftreten und darum schwer zu finden sind. SEILER (op. c.) hat die diploiden Rassen von *Solenobia triquetrella*, sowohl die bisexuelle als die parthenogenetische, in der Schweiz nur an wenigen Orten gefunden — alle in Gebieten gelegen, die entweder als Nunataker aus dem einstigen Vereisungsgebiet der Alpen aufragten oder in dessen Aussenrandzonen gelegen waren — während die tetraploide parthenogenetische Rasse weit verbreitet ist. SEILER hält die diploide bisexuelle Rasse für ursprünglich. Aus ihr ist zuerst die diploide parthenogenetische und aus dieser wiederum die tetraploide parthenogeneti-

sche Rasse entstanden. Es ist SEILER (1942, S. 517—518) bei seinen Versuchen in der Tat gelungen, aus der bisexuellen Rasse eine parthenogenetische hervorgehen zu lassen. Er hat neuerdings (1943, S. 696) auch eine Rasse gefunden, bei der die Parthenogenese fakultativ ist. Die diploide parthenogenetische Rasse ist also bei *Solenobia* nur eine kurzfristige Zwischenstufe, über welche aus der bisexuellen Rasse eine tetraploide parthenogenetische entstanden ist.

Bei den Rüsselkäfern kann es sich möglicherweise ähnlich verhalten. Es ist möglich, dass bei den Curculioniden auch diploide parthenogenetische Rassen vorkommen oder vorgekommen sind, wenn auch in beschränkten Gebieten. FAGERLIND (1944, S. 192—193) bemerkt, dass dieser Umstand, »dass vielen apomiktischen Polyploiden nicht apomiktische oder nicht einmal sexuelle Diploide entsprechen, kann auf einer reinen Konkurrenzerscheinung beruhen. Ist eine Entsprechung vorhanden, so macht diese oft den Eindruck, von Reliktcharakter zu sein. Sie ist auf ein kleines Verbreitungsgebiet beschränkt und weist oft deutliche Zeichen auf, dass sie in Rückgang begriffen ist, während die apomiktische Polyploide einen vitaleren Eindruck macht und ein relativ grösseres Verbreitungsgebiet hat«. Demnach liegt ja grosse Gefahr vor, dass »zunächst die diploiden Apomikten und dann auch die sexuellen Formen aussterben. Man hat daher wenig Möglichkeiten, sie anzutreffen«. Meines Erachtens wären die eventuellen diploiden parthenogenetischen Curculionidenrassen dem *Solenobia*-Fall entsprechend in denselben Gegenden zu finden wie die bisexuellen Rassen, also in erster Linie in mittel- und südeuropäischen Gebirgsgegenden. Die zytologischen Verhältnisse bei *Polydrosus mollis* (SUOMALAINEN, 1940 a und b) zeigen jedenfalls, dass es unter den Rüsselkäfern wirklich auch diploide parthenogenetische Formen gibt.

Es ist jedoch zu bemerken, dass der Übergang von der bisexuellen Rasse zur parthenogenetischen bei den Curculioniden ganz andersartige Veränderungen im Chromosomenmechanismus als bei *Solenobia* voraussetzt. Die Chromosomenverhältnisse von *Solenobia triquetrella* (SEILER, op. c.) sind derart, dass der Übergang zur parthenogenetischen Vermehrung verhältnismässig leicht ist. Die Eier auch der parthenogenetischen Rassen dieser Art machen nämlich zwei Reifungsteilungen durch. Die normale Chromosomenzahl wird dadurch wiederhergestellt, dass nach den ersten Furchungsteilungen zwei Kerne miteinander verschmelzen. Die bei der Befruchtung vor sich gehende Verschmelzung der Kerne der verschiedenen Individuen, die Amphimixis, wird bei der Parthenogenese von *Solenobia* durch die Verschmelzung der Kerne des-

selben Individuums, durch Automixis, ersetzt. Die tetraploide Rasse ist wiederum aus der diploiden parthenogenetischen gewiss auf die Weise entstanden, dass bei den ersten Furchungsteilungen nicht nur ein, sondern zwei aufeinanderfolgende Kernverschmelzungen eingetreten sind. Bei den Curculioniden liegt die Sache anders. Weil bei ihnen die parthenogenetischen Eier nur eine Reifungsteilung, die Äquationsteilung, durchmachen, fallen bei ihnen, wenn die Vermehrung parthenogenetisch wird, die Tetradenbildung wie auch die Chromosomenreduktion gänzlich aus.

Nehmen wir an, dass auch bei den Curculioniden zuerst diploide parthenogenetische Rassen entstehen, so könnten sich aus jenen natürlich tetraploide durch Verdoppelung der diploiden Chromosomengarnitur entwickeln. Die Entstehung triploider und pentaploider Rassen dagegen ist auf diesem Wege schwer zu verstehen. Bei der Erörterung der Entstehung triploider Rassen könnte man ausser von einer diploiden auch von einer tetraploiden parthenogenetischen Rasse ausgehen. Es ist zu bemerken, dass (Näheres auf S. 438) die Metaphasechromosomen in den Eiern mancher polyploiden Curculioniden oft nicht in einer, sondern in mehreren Platten auftreten, wenn auch derart, dass in jeder Platte im allgemeinen ein oder mehrere volle Genome vorkommen. Da diese Teilplatten ziemlich weit voneinander entfernt liegen können, wäre es denkbar, dass irgendein Genom von der aus den übrigen Chromosomen bestehenden Gruppe und somit auch von dem Kern des mit seiner Entwicklung einsetzenden Eies endgültig ausgeschlossen würde. Könnte man doch dadurch von einer tetraploiden Rasse leicht zu einer triploiden gelangen.

Gegen eine solche Möglichkeit spricht jedoch ein schwerwiegender Umstand. Wenn das wirklich geschähe, so müsste es z. B. von *Otiorynchus dubius* in Finnland sowohl triploide als tetraploide Individuen geben, da etwa 45 % von den Eiern der finnischen Individuen Teilplatten enthalten. Es sind aber alle bisher von mir untersuchten finnischen *O. dubius*-Individuen durchgehends tetraploid gewesen. Die Entstehung triploider Rassen aus tetraploiden durch Teilplattenbildung ist also nicht wahrscheinlich. Der Umstand, dass *O. pupillatus*-Eier mit weit voneinander liegenden Teilplatten (S. 439) zu degenerieren beginnen, deutet darauf hin, dass solche Eier nicht entwicklungsfähig sind.

Es wäre auch denkbar, dass eine triploide Rasse durch Kreuzung zweier anderer Rassen entstehen könnte. SEILER (op. c.) ist es gelungen, die diploide bisexuelle und die tetraploide parthenogenetische Rasse von *Solenobia triquetrella* miteinander zu kreuzen, wobei triploide Indivi-

duen entstanden. Bei *Solenobia* kann jedoch wegen der weiblichen Heterogamietie der Schmetterlinge keine beständige triploide Rasse entstehen, denn die triploiden Individuen sind infolge des veränderten quantitativen Verhältnisses zwischen Geschlechtschromosomen und Autosomen intersexuell.

SEILER (1945) hat neuerdings auch ein triploides intersexuelles Freilandtier von *Solenobia alpicolella* gefunden, das nach ihm am wahrscheinlichsten als Nachkomme eines überreifen begatteten Weibchens entstanden ist. In den Eiern der überreifen bisexuellen Weibchen wird nämlich die Chromosomenzahl möglicherweise durch Automixis zur Diploidie aufreguliert; wird weiter ein solches Ei bei der Ablage besamt, so resultiert die Triploidie und damit Intersexualität.

Weil bei den Curculioniden das Weibchen homogametisch ist, sind die triploiden Weibchen nicht intersexuell. Somit können bei ihnen beständige triploide parthenogenetische Rassen vorkommen. Doch kann bei den Rüsselkäfern eine triploide Rasse als ähnliche Kreuzung wie bei den Solenobien nicht entstehen. Weil die Eier aller *Solenobia*-Rassen zwei Reifungsteilungen durchlaufen, so sind die Eier eines tetraploiden *Solenobia*-Weibchens diploid; wird ein solches Ei besamt, so entsteht eine triploide Zygote. Die Eier der parthenogenetischen Curculioniden machen dagegen nur die Äquationsteilung durch; in ihren Eiern findet also keine Chromosomenreduktion statt. Bei etwaiger Kreuzung einer tetraploiden parthenogenetischen Rasse mit einer diploiden bisexuellen müsste also eine pentaploide Rasse entstehen. Eine solche wurde ja bei *Barynotus moerens* in den Österreichischen Kalkalpen gefunden (S. 437). Eine triploide Rasse könnte dagegen aus der Kreuzung einer diploiden parthenogenetischen Rasse mit einer diploiden bisexuellen entstehen, denn dabei vereinigten sich eben ein diploides Ei und eine haploide Samenzelle.

Das Vorhandensein pentaploider Rassen bei den Curculioniden deutet darauf hin, dass die parthenogenetischen Rassen sich in der Natur wirklich mit den bisexuellen kreuzen können. Die Entstehung einer pentaploiden parthenogenetischen Curculionidenrasse ist nämlich am wahrscheinlichsten auf die Befruchtung eines tetraploiden Eies zurückzuführen (vgl. z. B. MÜNTZING, 1944, S. 633; FANKHAUSER, 1945, S. 36), und dies wäre am leichtesten eben bei der Kreuzung einer tetraploiden parthenogenetischen Rasse mit einer diploiden bisexuellen denkbar. Und könnte sich einmal die diploide bisexuelle Rasse mit polyploiden parthenogenetischen Rassen kreuzen, so wäre eine Kreuzung der

beiden diploiden Rassen, der bisexuellen mit der parthenogenetischen, natürlich eher möglich.

Die relative Häufigkeit der triploiden parthenogenetischen Rassen — von den von mir untersuchten parthenogenetischen Curculionidenrassen oder -arten ist ja eine diploid, 11 sind triploid, 4 tetraploid und eine pentaploid — zeugt dafür, dass die Triploidie leichter als die höheren Polyploidiestufen entsteht. Dies könnte möglicherweise eben zum Teil darauf beruhen, dass die beiden diploiden Rassen, weil sie in denselben Gegenden vorkommen, bessere Voraussetzungen zur Kreuzung miteinander haben als eine diploide bisexuelle und eine polyploide parthenogenetische, die oft verschiedene Gegenden bewohnen.

Der Umstand, dass die höheren Polyploidiestufen bei den Curculioniden verhältnismässig selten sind, kann wenigstens zum Teil auch davon herrühren, dass entwicklungsphysiologische Bedingungen die obere Grenze der Polyploidiestufe ziemlich niedrig halten.

Bemerkenswert ist, dass der Kreuzung verschiedener Rassen auch keine morphologischen Hindernisse im Wege stehen, denn SZÉKESSY (1937, S. 579—581) hat festgestellt, dass der Bauplan des weiblichen Kopulationsapparates wenigstens einiger polyploiden parthenogenetischen Curculionidenformen (z. B. *Otiorrhynchus ovatus*) »keine sekundären Modifikationen aufweist, sondern dass er trotz der abgeänderten Fortpflanzungsweise seine ursprüngliche Ausgestaltung beibehalten hat«. Ferner ist zu bemerken, dass die jungen weiblichen Imagines bei sowohl bisexuellen als parthenogenetischen Curculioniden in ihren Ovarien noch nicht reife Eier haben. Somit hätten auch die parthenogenetischen Weibchen vor der Eiablage genug Zeit, mit Männchen der bisexuellen Rasse zu kopulieren. Was die sich eventuell kreuzenden Rassen betrifft, könnten m. E. als solche ausser den verschiedenen Rassen derselben Art vielleicht auch solche nahestehender Arten in Frage kommen. Im letzteren Falle hätten wir es nicht mehr mit Autopolyploidie, sondern mit Allopolyploidie zu tun.

Eine zweite, von der im vorhergehenden erörterten prinzipiell ganz verschiedene Möglichkeit, die Entstehung der polyploiden parthenogenetischen Curculionidenrassen zu erklären, wäre die Annahme, dass die Parthenogenese und die Polyploidie bei den Curculioniden gleichzeitig entstehen. Bei den Amphibien ist festgestellt worden (Näheres bei FANKHAUSER, 1942 und 1945), dass extreme Temperaturbedingungen — sowohl nach oben als nach unten hin — in den abgelegten, sich im Metaphasestadium der 2. Teilung befindlichen Eiern die 2. Reifungsteilung verhindern, wodurch diploide Eier entstehen. Wenn solche Eier

befruchtet sind, entwickeln sie sich zu triploiden Tieren. So kann z. B. rasche Temperaturerniedrigung während der Laichzeit dazu führen, dass auch in der Natur triploide Individuen entstehen.

FANKHAUSER (op. c.) hat auch einige tetraploide und einige pentaploide Urodelenlarven gefunden. Die Entstehung der ersteren könnte nach ihm (1945, S. 42) so erklärt werden, dass die extremen Temperaturbedingungen die Trennung der Teilungsprodukte der Chromosomen in der 1. Furchungsteilung des normalen befruchteten Eies verhindern, wodurch die diploide Chromosomengarnitur verdoppelt wird. Ein pentaploides Tier wiederum könnte durch Befruchtung eines tetraploiden Eies entstehen. »A tetraploid gamete may originate in various ways, e. g., by suppression of both maturation division and inclusion of all tetrads in a single nucleus» (1945, S. 36). Bei den Amphibien führt die Polyploidie jedoch nicht zu Parthenogenese.

Es ist nicht ausgeschlossen, dass auch bei den Curculioniden etwas Ähnliches geschieht. Die Entstehung polyploider parthenogenetischer Curculionidenrassen als Folge solcher Ereignisse setzt jedoch notgedrungen voraus, dass gleichzeitig mit der Polyploidie eine solche Genkombination entsteht, die sowohl das Ausbleiben der Tetradenbildung und der Chromosomenreduktion, also das Ausbleiben der einen von den zwei Reifungsteilungen, als die parthenogenetische Entwicklung des Eies verursacht. Es ist zu bemerken, dass die unbefruchteten Eier des Seidenspinners, *Bombyx mori*, nach einer kurzen Wärmebehandlung sowohl nur eine einzige Reifungsteilung durchlaufen, die eine Äquationsteilung ist, als sich parthenogenetisch entwickeln (ASTAUROV, 1940).

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### SUMMARY.

The present investigation deals with the chromosome relations of thirteen bisexual and ten parthenogenetic weevils, Curculionidae.

All the investigated bisexual species — they all belong to the genus *Otiorrhynchus* — have the same chromosome number ( $2n = 22$ ). The males are heterogametic, belonging to the XY type; the females are homogametic. In all species bivalent formation takes place normally. In them, chiasmata are clearly visible and become symmetrical and terminalized simultaneously with the contraction of the chromosome. Consequently, typical rod-bivalents with one terminalized chiasma, and ring-bivalents with two chiasmata, are formed.

All the parthenogenetic weevils dealt with here are polyploid. Among them there are triploid, tetraploid and pentaploid species. *Otiorrhynchus niger* (from the Austrian Alps), *O. scaber* (from the Austrian Alps), *O. singularis* (from Helsinki and Berlin), *O. salicis* (from the Austrian Alps), *O. sulcatus* (from Helsinki), *O. gemmatus* (from the Austrian Alps), *Sciaphilus asperatus* (from Berlin) and *Strophosomus melanogrammus* (from Berlin) are triploid. *Otiorrhynchus pupillatus* (from the Austrian Alps) is tetraploid and *Barynotus moerens* (from the Austrian Alps) pentaploid. The egg-cells pass through one maturation division only, which is equational. In most parthenogenetic species the chromosomes at the metaphase of the maturation division may be arranged in two or even three different plates, each plate containing one or more complete sets of chromosomes.

Including the weevils studied by the author previously (SUOMALAINEN, 1940 a and b) the chromosome number of 17 parthenogenetic species and races is known. Of these 1 is diploid, 11 triploid, 4 tetraploid and 1 pentaploid.

Three species, viz. *Otiorrhynchus niger*, *O. salicis* and *O. gemmatus*, occur in the Austrian Alps (in Lunz am See) as well as a diploid bisexual as a triploid parthenogenetic race.

Of *Otiorrhynchus scaber*, a triploid parthenogenetic race is met with in the Austrian Alps and a tetraploid parthenogenetic race in Finland. It is possible that the former is distributed over Central Europe (at least in mountains) and the latter over North Europe. Of this species,

too, a bisexual, probably diploid race is known from some localities in the East Alps.

Of such *Otiorrhynchus* species as occur in the Alps both as a bisexual and a parthenogenetic race, the bisexual race is usually met with in habitats which were free from ice during the Würm Glaciation («Massifs de refuge»). The parthenogenetic race, again, is distributed in areas which were then covered with ice. This depends at least mainly on the polyploidy of the parthenogenetic races. The same cause has also been active elsewhere in giving the parthenogenetic races a distribution different from that of the bisexual races.

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# STUDIES ON THE CAMPHOR REACTION OF YEAST

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## I. INTRODUCTION.

IT has long been known that various *Saccharomyces* species under certain conditions may change their normal mode of growth and propagation. Instead of a bud growing out, which is abstricted after having reached a certain size, an oblongate tube is formed which is not separated from the mother cell. Brewers and technical workers have often observed such so-called involution forms in aging yeast cultures in connection with the autolysis phenomenon.

One factor which has been found to be of importance for the cellular shape of yeast is the temperature. Usually it has turned out that colder conditions favour a longer and narrower cell-form, while warmth causes the cells to become rounder and isodiametric. HANSEN (1886) shows that low temperature may give rise to associations of mycelium-like cells in such *Saccharomyces* species as, under high temperatures, give rounded, solitary cells. ZIKES (1920) studies the influence of temperature on different yeast genera and observes changes in cell-shape, »Modifikationen, d. h. flüchtige Varietäten», if the yeast is cultivated under abnormal temperatures. These aberrants disappear again after a short period of optimal temperature. He points out that the typical reaction is that at low temperature the yeast partly acquires a longer cell-shape, partly shows a tendency to hang together in colonies.

Certain yeast species are also characterized normally by long and narrow cells which preferably grow in colonies. Also within species which normally have a rounded cell-shape and live as solitary cells there may be found genotypes with greater or less tendency to grow like the former type. Thus, in *Saccharomyces anomalus*, by selecting one hypha-bearing cell BARBER (1907) succeeded in breeding a strain which continued to maintain this quality. The cells formed hyphae at the end of which normal cells could again bud off.

On comparing haploid and diploid yeast of the same genotype LINDEGREN (1944) found that the haploids showed greater tendencies

to an extended hypha-like growth: »Extremely thin elongated cells are also found in haploid cultures. Much of this variation is simply due to environmental conditions. Both bloated cells and long, thin cells are generally characteristic of haploid cultures. Many haploid cultures show a predominance of one or the other type, but these types are generally absent from diploid cultures» (l. c., p. 157).

SEGAL (1938, 1939) observed a regular appearance of morphological aberrations in the yeast cells after treatment with fusel oil and certain higher alcohols. He describes abnormal budding giving rise to cell-chains, where mother cells and daughter cells do not separate, and also deals with the origin of oblongate cells, beacher-like cells, etc.

The first worker to be able experimentally to produce a high and regular frequency of these aberrant involution forms is BAUCH (1941). He describes the peculiar behaviour of the yeast cells under influence of camphor as follows: »Lässt man Campherdämpfe auf die Hefe einwirken . . ., so zeigt sich bei geeigneter Dosierung ein überraschender morphogenetischer Einfluss dieser Substanz. Nach anfänglicher Wachstumshemmung beginnt ein Teil der überlebenden Zellen keimschlauch-ähnliche Auswüchse zu bilden, die schliesslich zu kleinen, meist bizarren Mycelien auswachsen . . . Die Reaktion wird sowohl vom rechtsdrehenden natürlichen Campher, wie vom racemischen synthetischen Campher ausgelöst. Dagegen sind alle bisher untersuchten Campher-derivate und dem Campher nahestehenden Verbindungen wirkungslos geblieben» (1943 a, p. 46).

By taking progeny from camphor-treated cells BAUCH has also succeeded in producing giant and super-giant types of yeast, which he considers to be polyploids. Although he has not obtained the typical camphor reaction with any other substances, he reports the induction of polyploidy after manifold different treatments with c-mitotic, carcinogenic and growth-promoting substances. In a later paper (1943 b) BAUCH shows the origin of constant giant races after radium irradiation as well.

With the use of BAUCH's technique SUBRAMANIAM (1945) induced mycelium growth in yeast and also obtained tetraploid and octoploid cells. THAYSEN and MORRIS (1943) have induced giant growth and probable tetraploidy in *Torulopsis utilis* by using camphor. They also found a similar response after treatment with some other camphor derivatives.

Since 1942 a co-operation has been going on between the Central Laboratory of the Stockholm Brewery Co. and the Cyto-genetic Labor-

atory of the Swedish Seed Association. Special attention has been directed to the camphor reaction and the conditions for the origin of natural involution forms. Thus, LEVAN and SANDWALL (1943) determined the effective concentrations of camphor and borneol in giving camphor reaction. Later on I tested a fairly large number of chemicals as to their »camphor activity». Although great differences in efficiency were found between different substances, I found some activity in almost all of them, independently of their chemical constitution. Various circumstances, however, such as lethality and toxicity often make the demonstration of the reaction difficult. A short report (LEVAN, 1944) gives some data concerning the camphor activity of aliphatic alcohols. This paper is an attempt to put the camphor reaction into its wider connection with other similar vital reactions.

Studies on this problem have gone on for different periods. The experimental work has been done at the Central Laboratory and the cytological analysis at Svalöf. My conclusions of 1944 have gained further support. The present paper will give the material underlying the conclusions, as far as it has appeared up to now. The empirical rule which various workers have found to be valid in toxic and narcotic reactions, viz. a correlation between certain physical properties of the substances and their activity, has been studied in my experiments on yeast.

The present work is based on experimental series with a certain yeast clone, viz. the commercial variety of beer yeast (bottom yeast) of the Stockholm Breweries. As culture medium use has been made of the standardized type of wort generally used at the Central Laboratory. The possibly somewhat unfavourable condition of using wort instead of a synthetic culture medium in this kind of work was thought to be counterbalanced by the great advantage that the normal behaviour of the yeast in question in this wort type was studied and well-known long ago and that this wort is known to furnish optimal conditions for the yeast. In each substance tested the yeast was treated with a series of concentrations from saturated solution down to such dilutions as do not give any visible effect. In some series the rather comprehensive manner of proceeding described by LEVAN and SANDWALL (l. c.) was employed, each treatment being made in 100 cc of wort, the cell numbers being counted on different occasions in BÜRKER's counting chamber. Many experiments were more orientating and were made in wort quantities of 10 cc in small FREUDENREICH flasks. The poison effect was often estimated qualitatively without any

exact counts. It was realized that such estimations could be made fairly correctly if slides with the treated yeast were compared with slides from the control cultures. Exact cell numbers given below are always based on countings in BÜRKER's chamber,  $\frac{1}{10}$  mm<sup>3</sup> having been counted on each occasion. When determining the frequency of camphor colonies each colony was recorded as one »individual» irrespective of its cell number.

Of certain substances that did not give lethal effect in saturated solution even solutions with various excesses of undissolved substance were tested. It was already known from our treatments with camphor and borneol (LEVAN and SANDWALL, l. c.) that an increased amount of precipitate may give an increased effect of both camphor reaction and toxicity. Similar conditions are also found in certain c-mitotic treatments (LEVAN and ÖSTERGREN, 1943, pp. 389—390).

The present investigations have been financed by the Stockholm Brewery Co., and the co-operation between the Central Laboratory and the Cyto-genetic Laboratory has been constantly supported by Dr. HARRY LUNDIN, Scientific Leader of the Stockholm Brewery Co., and Professor ÅKE ÅKERMAN, Director of the Seed Association. The staff of the Central Laboratory has in every way helped my work, and I am especially indebted to Mr. C. G. SANDWALL, microbiologist, and Mr. E. SANDEGREN, chief chemist of the laboratory. Drs. E. STEINEGGER and G. ÖSTERGREN have read the manuscript.

## II. THE MORPHOLOGY OF THE CAMPHOR REACTION.

### 1. CELLULAR MORPHOLOGY.

The opinion of BAUCH that the camphor reaction is a property peculiar to camphor has not been found to hold true. The reaction is induced by most organic substances tested, aliphatic, alicyclic, monocyclic aromatic. In my experiments bicyclic and polycyclic aromatic compounds, among which, it is true, only few have been tested, have been without visible effect. The general type of the reaction is subject to considerable variation between different substances and groups of substances. It only rarely grows as complete and extreme as in camphor.

In accordance with the practice at the colchicine reactions, which are induced by a great many substances besides colchicine but are nevertheless suitably called c-mitosis and c-tumour reaction, I will hereafter refer to the present reaction as *the camphor reaction* or, abbreviated, *the cf-reaction* even when it is caused by other substances

than camphor and when its type deviates from the typical camphor effect in narrow sense. I will thus discuss cf-forms, cf-cells, cf-colonies, cf-tendencies, cf-activity, and so on.

The common feature in the complex of deviations from the normal cell growth induced by the cf-reaction is the condition that the bud cell does not as normally loosen from the mother cell but grows further still connected with the mother cell. Under the influence of cf-substances, consequently, associations of cells are formed which cannot be brought apart even by heavy shaking of the culture bottle. These associations may in extreme cases grow out into macroscopically visible cf-colonies with thousands of cells (see, for instance, LEVAN and SANDWALL, 1943, the microphoto Fig. 1 *h*). The yeast actually changes into a colony-forming organism.

This hanging together into colonies is no doubt caused by a dissimilarity in the development of the bud cell of the cf-forms as compared with normal budding. This deviation in the formation of the bud cell is striking in extreme cases. Fig. 1 *d, f* shows the budding of an untreated cell. The canal between the mother cell and the bud rapidly narrows down into a thin passage. Fig. 1 *i—l* pictures the same stages under the influence of camphor. The corresponding canal is often broader and in extreme cases (Fig. 1 *u, y*), there is no constriction at all. The new cell is then started as a hypha-like tube, in the interior of which transverse cell-walls may later on develop.

Under advanced narcosis the tube may grow on apically into a long, wide hypha. This may later be divided by cell-walls into an irregular row of cells. Camphor, tertiary butanol and a few other substances cause the development of such formations. Most substances, however, do not narcotize the growth control so deeply. It may be noticed that the tubes make attempts to delimit single cells during their growth. It consequently originates numerous shallow constrictions of the tube, which will show a more or less regular undulation of its outline. Propanol has in my experiments given especially pronounced undulations (Fig. 3: 21—23). The portions of the tubes delimited by the constrictions may later on form separate cells and may even be liberated from the colony. After certain treatments the tube may grow forward under iterated turns to both sides, thus developing a more or less regular zigzag tube or in some cases a clear spiral tube. Such tubes are formed by normal butanol (Fig. 3: 34—37). More irregularly growing cf-tubes may form unilateral swellings, as sometimes observed in pentanol (Fig. 3: 51—52).

Besides the more tube-like cells there may be induced cellular growth in all directions, spherical, vesicular or pumpkin-like cells originating. Often the basic part of these cells is larger, which results in flask- or pear-like formations. Ether and acetone induce this type of growth (Fig. 4 *a—m*). These inflated cells may become very large and may alternate within the cf-colonies with thinner, tube-like cells. In some cases the cf-cells start as tubes, but are soon divided into smaller, rounded cells of a normal cell-shape. Chloroform, ethanol and urethane give such colonies (Fig. 3: 18, Fig. 4 *y*). They resemble bunches of grapes, and only in the outskirts of the colonies are tube-cells found.

The aromatic substances have in common the feature that the cf-reaction induced by them is hard to demonstrate. There seldom occur the long tubes which are typical of many aliphatic treatments. The cell form of cf-forms of benzene, for instance, is more or less rounded even if it is often tube-like from the beginning (Fig. 2).

It is of some interest to note that at the same time as the yeast changes into a colony-forming organism its individual cells disclose an evident bipolarity. The oldest or basal part of a cell thickens, and from this part only seldom do new tubes grow out. First the apical part of the cell grows on, thereafter new lateral tubes are initiated around the apex of the original cell. These lateral axes of the second order grow out, and from them later on tertiary axes are formed. A growth pattern of a certain regularity may be seen. Its type is similar to those met with in related colony-forming yeast species. Cf-forms of such regular constitution have been found after treatment with chloroform (Fig. 4 *p*) and chloral hydrate (Fig. 4 *r, s*). It must be pointed out, however, that this schematic pattern is found only in ideal cases, many irregularities occurring. Tubes may grow out also from the middle or basic part of the cells; the first cell influenced by the cf-reaction may grow out at both ends, resulting in a bilateral colony, or it may form several tubes, resulting in a more radiary colony.

I want to stress the extreme variability in type and the complex nature of the cf-reaction. If it is, as I assume, a narcosis of the normal growth, it may be as complicated as normal growth itself. The control of the many different functions which constitute the normal growth may become switched off in fractions different in different substances. When judging the individual cases it is necessary to take into account the relative position of the activity threshold for cf-action and the toxicity and lethality thresholds.

The c-tumour reaction of root tips makes an interesting parallel to the cf-reaction: in both cases it is a question of a disturbance of the normal cell growth. At the c-tumour reaction, cells which normally grow in length more than breadth will under the influence of narcotics grow more or less isodiametrically. The cf-reaction causes cells that are normally elliptic to grow out into cylinders or tubes. The c-tumour reaction also shows variations in its morphology, depending on which substance has been employed. Colchicine, for instance, brings about small well-defined tumours in *Allium* roots, while acenaphthene gives more undefined, gradually swelling tumours. As a comparison the manifold variations and nuances shown by different narcotics in their action on animals may also be recalled.

## 2. NUCLEAR MORPHOLOGY.

Owing to the drastic changes in cellular shape and cellular volume induced by the more efficient cf-substances it is to be expected that the nuclear conditions are also disturbed in the cf-cells. BAUCH makes comparisons between the effect of camphor with that of colchicine on higher plants. This is also justified on the basis of his results: he obtained now and then single colonies with larger cells, which afterwards maintained their larger cell-size through many generations. No investigations on the nuclear cytology of the cf-forms were made by BAUCH, however, nor have such studies been made elsewhere.

It may be mentioned, however, that SEGAL (1939) made some efforts to investigate the nuclei of his aberrant yeast cells induced by higher alcohols. He found abnormally shaped nuclei of fixed and HEIDENHAIN-stained material showing a folded or lobated shape (SEGAL, 1939; Fig. 8).

Since it has been shown that cellular divisions go on even after long treatment with cf-substances, it is *a priori* excluded that any permanent or complete colchicine effect can be imposed on yeast by camphor and the other cf-substances. C-mitosis holds up the nuclear divisions and as a consequence also the cellular divisions.

As I considered it a vital point in these investigations to be able to observe directly the behaviour of the nuclei, I have devoted much work on attempts to bring about analysable nuclear stainings in yeast. Using the technique of WINGE and LAUSTSEN (WINGE, 1935, pp. 104—105), I tested different fixatives and different pre-treatments. I found CARNOY, BENDA, FLEMMING, MERKEL, NAVASHIN and ZENKER capable of giving fairly good results. The pre-treatment with sodium hydroxide recom-

mended by WINGE may improve the results considerably. FEULGEN gave the securest results as a stain, but crystal violet and haematoxyline could also give favourable stainings. FEULGEN followed by staining in gentian violet may give quite good contrast, the nuclei being stained red violet while volutine granules and other stainable components of the cell take a pure blue stain.

The fixation and staining both of untreated yeast and of cf-cells are, however, always whimsical. The casual state of the culture and other factors out of control seem to play an important rôle. After a great deal of work it was evident, however, that the yeast nuclei consist of a number of distinct bodies, which go through the mitotic stages in the same manner as chromosomes. Especially at late metaphase individual chromosomes may be visible in side view, their centromeres evidently not yet having been divided. One chromatid is then directed towards each pole, the centromeres seem to have more or less terminal position (Fig. 1 *e*, 2 *i*). The chromosomes at this stage resemble small meiotic bivalents. There is a strong tendency of the chromosomes at all stages to stick together into one or two bodies. The low chromosome number earlier published for yeast (e. g., BADIAN, 1937; SINOTO and YUASA, 1941) may have been influenced by such fusions. After the anaphase separation the two daughter groups of chromosomes tend to be strongly coloured, and each of them may form one or two distinct bodies that often give the impression of a chromosome number of two or four. I have sought very carefully for good metaphase plates, but even in well-stained material most metaphases are not analysable. Often the chromosomes form a ring on a hollow spindle (Fig. 1 *d*). The tendency of the chromatic material to gather in the periphery is found also in resting nuclei (Fig. 2 *c*, *f*).

Not even in the best metaphase plates I have come across (Fig. 1 *a—d*) is it possible to give an exact chromosome number. Some facts seem certain, however. The chromosome number is higher than has been generally assumed, ten being a minimum number. Size differences among the chromosomes are often noticed. In some slides two chromosomes seem larger than the rest (Fig. 1 *c*). In other slides, however, very clear plates have been seen without these two large chromosomes. Probably the size of the chromosomes is liable to variations owing to variable action of the pre-treatment in NaOH. The size of the smallest observable chromosomes is at the limit of what is visible in the microscope (about  $0.1\ \mu$ ). It is quite possible that more of the small chromosomes are present than I have been able to demonstrate.

It is also possible that the larger chromosomes are built up from smaller ones sticking together. In the size-class of these chromosomes, and with the rather faint stainings even at their best, the details seen must always be taken with great caution.

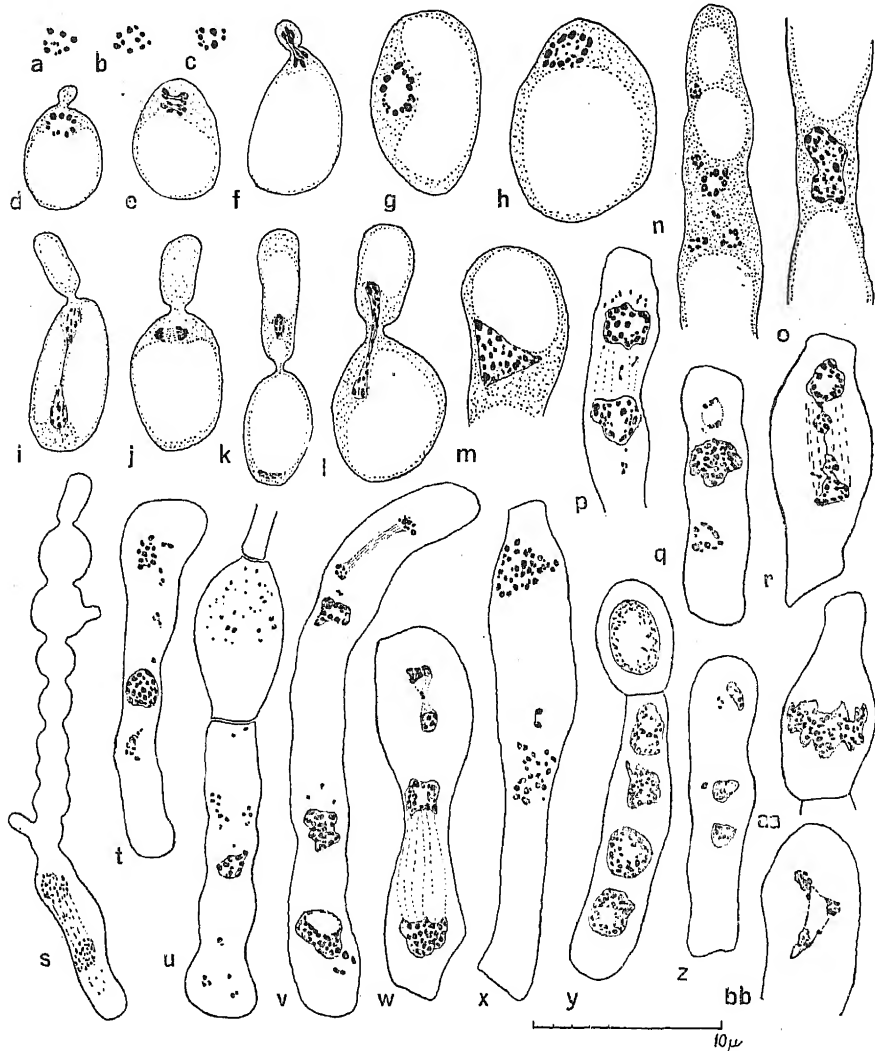


Fig. 1. The caryology of normal yeast and cf-forms. *a-c*: untreated yeast; *f-o*: yeast grown on wort-agar with camphor content; *p-bb*: yeast grown in wort with tertiary butanol; *a-d*: normal metaphases, *e* normal anaphase; *f-l*: various mitotic stages in giant cells; *g-h*: metaphases with doubled chromosome number; *m-bb*: various nuclear disturbances found during advanced cf-growth; *a-o*: FEULGEN, *p-bb*: acetocarmine. —  $\times 2500$  (*s*:  $\times 1200$ ).

The nuclear conditions of the cf-forms were first studied on yeast grown on agar prepared from wort solutions of known camphor content. The outgrowing giant colonies were smeared on slides and fixed and stained in the same way as untreated yeast. In the giant colonies thus produced the majority of cells are always found to be of the normal type, but they are intermingled with a certain frequency of cf-cells. Most of the cells have normal nuclear conditions. About the same chromatin amount is present as in untreated material. The separate chromosomes may be estimated to ten in the camphor-treated cells also.

After such treatment, however, it is noticed that the cell-size varies more than usual even among the normally formed cells. I found that some of the large cells had larger nuclei than normally, and in some favourably fixed giant cells it was possible to demonstrate the doubled chromosome number of at least 20 (Fig. 1 *g, h*). These are evidently chromosome-doubled cells, and the chromosome doublings are independent of the tube-like cf-growth. Such cells look quite viable; no doubt they may give rise to tetraploid strains if isolated. In the cell population of the giant colony, however, the tetraploid cells are in such low frequency as to make it very improbable that they can stand the competition with normal diploid cells. The mechanism of their doubling cannot be known with certainty. The tetraploid cells have normal mitosis with full spindle activity. If they have been induced by c-mitosis, this must have been transient, only affecting one mitotic cycle. Since it is known that the yeast has the ability of rapidly acquiring a certain resistance towards cf-treatments, especially if the concentration of the active substance is immediately above the threshold value, it is perhaps not absolutely inconceivable that one mitosis may become narcotized, but that the cell after that again takes command over its mitotic mechanism, the subsequent mitoses being normal. The other possibility for the origin of tetraploidy is an endomitotic process. Unfortunately, the nature of the material impedes any close study of the details of these processes, so a decision on this point must rest so far.

The main interest in this material centres in the real outgrowing cf-forms. In the beginning, during the first week of treatment, the mitoses of even extreme cf-cells are seen to proceed normally, spindle disturbances being exceptions. In many cases it was possible to estimate the chromosome number within the cf-forms. Usually it was normal diploid. Often it occurred that the nuclei of the cf-cells were more diffuse than neighbouring normal cells, they took the stain more faintly and, generally speaking, showed indications of being in a deeper resting

condition than the other surrounding cells. This may suggest the possibility of an endomitotic mechanism. When the chromosomes are again stainable, there is often found in the more advanced cf-forms a higher chromosome number than normally, 4x, 8x or still higher. Usually the spindles are functioning normally. Only in a few cases (Fig. 1 *m—o*) were there found indications of clear spindle disturbances, such as several nuclei lying close together and clearly showing variable chromosome number.

In the autumn of 1943 I made a number of acetocarmine slides of yeast grown 2 or 3 weeks on wort with different cf-substances. These slides contained a great frequency of much more advanced cf-forms than the above FEULGEN-slides. The acetocarmine slides were stored, which could be done because the acetocarmine employed contained glycerol. In 1946 I re-investigated these slides, finding, as might have been expected, that the cells had taken so much stain as to be quite blackish-purple. In some of them, however, I faintly saw some darkly stained bodies resembling nuclei. I accordingly put the slides in 45 % acetic acid in order to wash out the excess of stain. Thereafter the slides were made permanent by mounting in canada balsam. It was found that the cell form had remained almost unchanged during this procedure. In some of the slides fairly good nuclear stainings were present. Especially one slide, treated a fortnight with 0,2 mol/l tertiary butanol, showed excellent staining. In this slide I learned more about the chromosome conditions of the cf-forms than in the several hundreds of earlier FEULGEN-slides, although, broadly speaking, the nuclear disturbances met with in this acetocarmine slide agreed with those earlier studied. They were, however, more frequent and more extreme.

The chromosomes were undoubtedly somewhat more swollen than after ordinary fixation and staining, which may not be entirely disadvantageous considering the small size of the chromosomes. The cells were also decidedly larger than after the other fixation methods. I found that I had interpreted correctly the chromosome form and size in the earlier slides. Here there were often found single chromosomes lying free in the plasm and furnishing a good opportunity to study the chromosome shape and size. Almost all cells in this slide represented very extreme cf-disturbances. The nuclear conditions were highly irregular, instances of cells from this slide being pictured in Fig. 1 *p—bb*. It was noticed that the synchronization between cellular growth and mitosis was often disturbed. Thus, in the periphery of the colonies

there were very often no nuclei present, although the constrictions of the cell-wall indicating the limits of the future cells were fully prepared. The mitotic activity was evidently lagging behind the cellular growth (Fig. 1 *s*). In the more centrally located cells, on the other hand, it was not unusual with four or more large nuclei in one cell (Fig. 1 *y*). The nuclear size was highly varying. Often two very large nuclei with many chromosomes in each and two decidedly smaller nuclei were present in one cell (Fig. 1 *w*). In this case it could be inferred from the mutual positions of the nuclei and from spindle fibres remaining between each pair of nuclei that the larger two represented telophase of one mitosis and the two smaller ones of another mitosis. The distance between the telophase nuclei was in this and in other cases roughly proportionate to their size, the larger nuclei lying decidedly more apart than the smaller ones. The spindle has perhaps a certain size relation to the number of chromosomes participating in a mitosis. Ordinarily all spindles were located in the longitudinal direction of the tube cells, but very small nuclei could develop their spindles transversally.

It has a certain interest here to be able to demonstrate that even at such an advanced stage of cf-growth the spindles function and do so rather regularly. The anaphases, it is true, all showed serious disturbances, lagging chromosomes, multipolarity, formation of restitution nuclei, and so on. Sometimes the entire nuclear material was scattered out over the whole cell (Fig. 1 *u*, the upper cell). In this case a decided tendency to an arrangement of the chromosomes in pairs could be seen. This cell might represent a c-metaphase, and the pairs may be c-pairs.

Since certain objections may be raised against the use of acetocarmine in the staining of yeast chromosomes, this stain not being so selective a nuclear stain as desirable, I decided to repeat the stainings of similarly treated cultures by the FEULGEN technique. In the summer of 1946 some cultures were put in various concentrations of tertiary butanol and of benzene. Samples of the cultures were centrifuged every second day during the treatment. FEULGEN slides were prepared from the sedimented cells. The slides of both types of treatment showed normal mitoses during the first ten days mostly, although the typical cf-forms occurred as usual. Not until the 12th and 14th day did distinct nuclear disturbances begin to be frequent. Especially some fixations from the 16th day of a culture treated with a strong benzene concentration (0,012 mol/l) gave an abundance of mitotic disturbances, allowing an analysis of the nuclear behaviour during the cf-growth. (Here as elsewhere in the present paper »the first day» refers to ob-

servations 20—24 hours after the beginning of the treatment, »the second day» means a treatment of two days, and so on.)

Fig. 2 shows some cells from this treatment. A microphoto of a

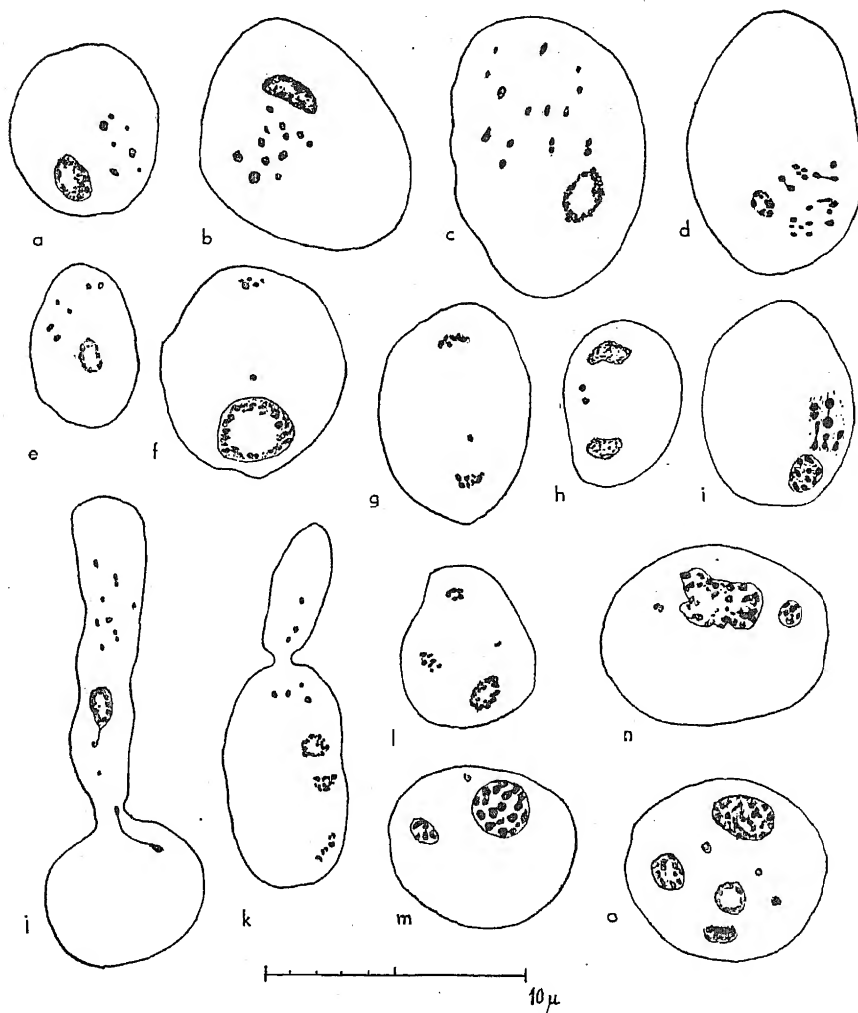


Fig. 2. Yeast cells treated for 16 days with 0,012 mol benzene, showing various mitotic disturbances, chromosomes scattered outside the nucleus (*a—e, j—k*), vagabond chromosomes (*f—h*), accessory spindle (*i*), multipolar telophase (*l—o*); FEULGEN. —  $\times 3500$ .

view field from a similar treatment has already been published in a preliminary communication to *Nature* (LEVAN, 1946). In this micro-

photo some of the commonest types of irregularities were gathered. It is seen from the present Fig. 2 that exactly the same type of disturbances are found here as in the aceto-carmin slide described above. The control in FEULGEN can thus be said to have strengthened the conclusions drawn above. Also here there occur nuclei of dissimilar size, some being many times the size of untreated nuclei. Often solitary bodies, strongly suggestive of chromosomes, are scattered outside the nuclei. What increases the impression of chromosomes is the often occurring clear doubleness of the bodies: it looks as if single chromosomes had divided in their abnormal position. In some cases two groups of chromosomes or two nuclei are situated one at each end of a cell with one single or one double body lying between them (Fig. 2 *g, h*), evidently laggards from the foregoing mitosis. Although the spindle structures are not stained by this technique, differences in refraction in some cases gave clear evidence of spindles. In Fig. 2 *i*, for instance, one spindle with five dividing chromosomes was observed outside a nucleus. Pictures such as Fig. 2 *l, o* are strikingly similar to multipolar telophases, such as are often seen in higher plants, for instance after a weak c-mitotic influence.

As already pointed out above, a special interest is attached to the solitary chromatic bodies lying outside the denser clusters of nuclear material. Benzene acts upsetting on the mitotic spindles, which under normal conditions keep the chromosomes crowded together. The result is that the chromosomes are scattered out, allowing the study of single chromosomes as well as of the two daughter halves of one chromosome. Thus, an opportunity is given, which seldom occurs normally in yeast, to observe single chromosomes. It appears that the chromosome size is decidedly smaller than has been assumed by those workers who have given a low chromosome number for yeast. The chromosome size established by studying this treated material is, instead, in good agreement with the size of the smallest single bodies found in the best fixations of untreated material. As mentioned above, in such material a chromosome number of at least ten was counted. While in this untreated material there was always a suspicion, even in the clearest cases, that these bodies were not entirely free from each other but might be chromomeres belonging to larger chromosomes, this benzene-treated material clearly shows that they really are free chromosomes, which may be present entirely separated from each other. The only other alternative is in my opinion that the treatment has effected

some fragmentation of chromosomes. This alternative seems less likely considering the regularity in the arrangement of the free bodies.

Summing-up the results of the caryological analysis of the cf-forms, it may be stated that the drastic changes in cellular shape induced by the cf-substances are also accompanied by considerable deviations from normal conditions in mitosis. There has been observed a highly increased chromosome number of individual nuclei. Mitotic disturbances, although rare in the beginning, later on occur regularly. Whether the increase observed in chromosome number is brought about by a transient c-mitosis or by an endomitotic process has not been decided.

### III. THE ALIPHATIC ALCOHOLS.

In this and following chapters (III—VI) a systematic account is given of the experiments performed with the various substances. When possible the threshold values of lethality, toxicity and cf-reaction are recorded at the end of the description of each substance. Characteristic morphological features in the development of the cf-forms are also mentioned. Each group of substances is concluded with a brief general discussion.

#### 1. METHANOL.

a. *Orientating experiment* (<sup>8</sup>/<sub>9</sub>, 43). — Concentrations: 0,01—2 mol/l. After 2 days the cells were still living in 2 mol, but no development occurred. In 1 and 0,5 mol almost exclusively cf-growth occurred after 20 hours, on the 2nd day 1 mol still held many cf-forms, but in 0,5 mol most cells were now normal. Weak tendencies to cf-reaction were perceived in 0,2 and 0,1 mol. All except 2 mol were in fermentation on the 2nd day.

b. *New series* (<sup>26</sup>/<sub>1</sub>, 44). — Concentrations: 0,1—2 mol. In this experiment true cf-forms were found only in 2 mol. Tendencies to cf-growth were also seen in 1 mol. 2 mol was not lethal, but the fermentation was very much retarded. On the 17th day this concentration was fermenting, however.

The cf-forms of this alcohol were not extreme (Fig. 3: 1—14). On the 2nd day the cells of the concentration 2 mol were narrow and oblongate. The buds were often tube-like. Later on the cells were often rounded off. The number of cells in each cf-colony was never especially high. In one count the colonies contained 2—10 cells

(Table 1). The outgrowing tubes often had a somewhat undulated outline (Fig. 3: 7, 8).

Tested zone: 0,01—2 mol  
 Lethality: None  
 Toxicity: 2 mol  
 Cf-reaction: (0,5—1)—2 mol.

## 2. ETHANOL.

a. *Orientating experiment* (<sup>8</sup>/<sub>0</sub>, 43). — Concentrations: 0,01—2 mol. In 2 mol the cells did not show any development during the experiment, this culture never coming into fermentation. 1 mol showed cf-growth, after 2 days rather large cf-colonies of 20—30 cells were seen. 0,5 and 0,2 mol also gave tendencies to cf-growth.

b. *New series* (<sup>20</sup>/<sub>1</sub>, 44). — Concentrations: 0,1—2 mol. The results were similar to the former ones, but the frequency of cf-forms was lower. The only concentration with any considerable frequency of cf-forms was 1 mol after 20 hours. After 2 days this culture had also passed over into a phase showing preponderantly normal cells. All except 2 mol came into fermentation in 2 days. 2 mol did not show fermentation during the entire experimental period, which was 15 days.

c. *Testing of the zone 1—2 mol* (<sup>31</sup>/<sub>1</sub>, 44). — Concentrations: 6, 7, 8, 9, 10, 11, 12 volume % (1,0—2,0 mol). In this series cf-forms were observed in the following treatments:

6 %	after 20 hours and 2 days
7 »	» 20 » , 2, 3 and 7 days
8 »	» 20 » , 2, 3 » 7 »
9 »	» 7 and 12 days

6—8 % thus gave cf-forms in the beginning of the experiment, 9 % gave cf-forms later on. 10—12 % showed no development during the 15 days of the experiment.

The general type of the cf-forms was similar to that of methanol (Fig. 3: 15—18). One long tube-like bud grows out without separating from the mother cell. Often this tube from the beginning or gradually develops a great many constrictions (Fig. 3: 16, 17), later on delimiting separate cells. In that way long rows of cells arise.

Especially in the higher concentrations a long period of treatment, 7 days or more, brings about the origin of large colonies of the grape-cluster type (Fig. 3: 18).

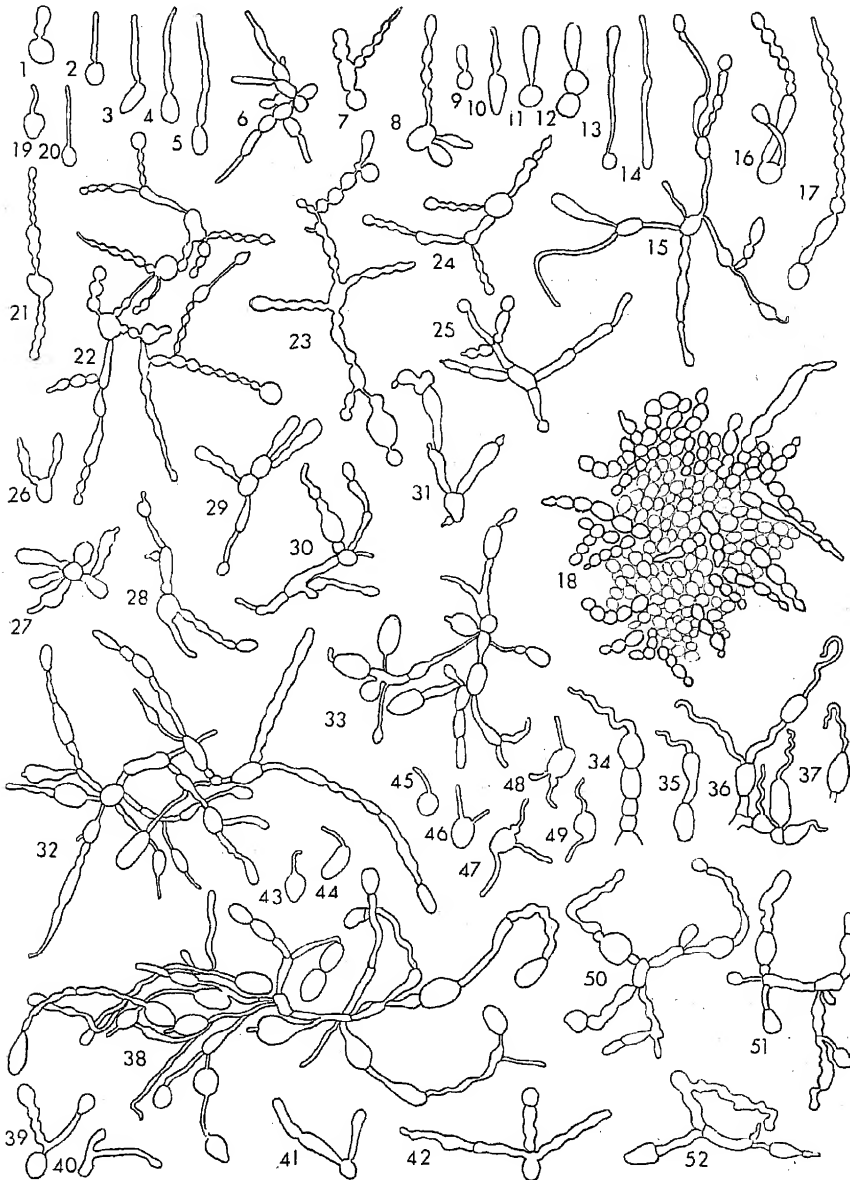


Fig. 3. Outline drawings of cf-forms induced by aliphatic alcohols: methanol (1—14), ethanol (15—18), propanol (19—25), normal butanol (26—37), iso-butanol (38), tertiary butanol (39—42), iso-pentanol (43—52). —  $\times 400$ .

Tested zone: 0,01—2 mol	Toxicity: 1—1,7 mol
Lethality: 1,7—2 »	Cf-reaction: 1—1,5 »

### 3. PROPANOL.

Both normal and iso-propanol were tested with concentration series from 0,02 to 2 mol (<sup>27</sup>/<sub>1</sub>, 44). Both showed little development in 1—2 mol. The iso-form seems to be a little less toxic than the normal form. In the former one cf-forms started growing out after 2 days in 1 mol, in the latter one no growth occurred in this concentration until after 7 days. 0,5 mol of the iso-form allowed full fermentation after 2 days, while 0,5 mol of the normal form gave no fermentation in 15 days. At that time it was not dead, however, but contained a decided cf-growth. In the normal form 0,2 mol is the highest concentration allowing fermentation in 2 days. A similar difference between the normal and the iso-form is apparent in the cf-reaction also. Cf-forms are found in 0,1—0,5 mol of the normal and in 0,2—0,5 mol of the iso-form.

The appearance of the cf-forms is characteristic (Fig. 3: 19—25). A maximum in the aliphatic series is here reached concerning the undulated outlines of the cf-cells. Complicated such types were found, for instance, in 0,5 mol of the normal form after 16 days (Fig. 3: 22).

	Normal	Iso
Tested zone:	0,02—2	0,02—2 mol
Lethality:	1 —2	2 »
Toxicity:	0,5	0,5 —1 »
Cf-reaction:	0,1 —0,5	0,2 —0,5 »

### 4. BUTANOL, C<sub>4</sub>H<sub>9</sub>OH.

a. *Normal, primary* (CH<sub>3</sub> · CH<sub>2</sub> · CH<sub>2</sub> · CH<sub>2</sub>OH), *orientating experiment* (<sup>8</sup>/<sub>9</sub>, 43). — Concentrations: 0,01—2 mol. In 0,5—2 mol the cells were killed. Cf-growth occurred in 0,05—0,2 mol. Thus, 0,1 showed after 2 days 100 % cf-forms, while 0,2 mol had only little development and 0,05 mol had predominatingly normal growth. The latter concentration had 100 % cf-growth after 20 hours.

b. *Normal, primary, new series* (<sup>28</sup>/<sub>1</sub>, 44). — Broadly speaking, similar results: 0,2 still had very little development after 6 days; probably most cells were killed. Cf-forms were found in 0,01—0,1 mol.

The cf-forms of this alcohol (Fig. 3: 26—37) were often large and contained irregularly shaped cells. Their general type is more extreme

than in lower alcohols. Large, rounded, oviform or piriform cells are formed, from which long hyphal threads take their origin. The distal tubes are not seldom turned in spirals, which may sometimes be surprisingly equal (Fig. 3: 34—37). If the origin of bead-like tubes indicates a series of intermittent impulses to normal growth, the spiral structure may indicate a more continuously acting asymmetric impulse.

c. *Normal, secondary butanol* ( $\text{CH}_3 \cdot \text{CH}_2 \cdot \text{CHOH} \cdot \text{CH}_3$ ) ( $^{19}/_6$ , 45). — Concentrations: 0,01—0,5 mol. While the cells in 0,5 mol did not show any development, 0,2 mol was in full fermentation after 2 days. Only 0,2 mol had any considerable number of cf-forms.

d. *Iso-butanol* [ $(\text{CH}_3)_2 \cdot \text{CH} \cdot \text{CH}_2\text{OH}$ ] ( $^{28}/_1$ , 44). — Concentrations: 0,01—2 mol. Conditions resembled those of normal butanol (above, b): 0,2—2 mol showed lethality. The toxic effect may have been somewhat less than in the normal form. Cf-forms occurred in 0,02—0,1 mol.

The cf-forms had the same type as in normal butanol, or were even somewhat more irregular (Fig. 3: 38). In the same colony large, inflated cells alternated with long thin tubes. It occurred that the tube-cell grew out from the middle of the mother-cell and not distally as usual. Sometimes two tubes grew out from the same end of one cell, as was observed to happen also in normal butanol (Fig. 3: 26). These V-shaped cf-forms are still more common in tertiary butanol (Fig. 3: 39—42).

e. *Tertiary butanol* [ $(\text{CH}_3)_3 \cdot \text{COH}$ ], *orientating experiment* ( $^8/_9$ , 43). — Concentrations: 0,01—2 mol. The cells were killed in 1—2 mol. 0,5 mol gave poor development, but small cf-tubes grew out in 2 days. They were narrow and twisted, and the cells showed poor viability. In 0,1—0,2 mol there was 100 % cf-growth after 20 hours. 0,1 mol already returned to mostly normal growth after 2 days. Single cf-forms also occurred now and then in 0,05 mol.

f. *Tertiary butanol, new series* ( $^{28}/_1$ , 44). — Same concentrations and, broadly speaking, same results as the foregoing. Cf-forms occurred in 0,02—0,5 mol, especially numerous and well-developed cf-forms were seen in 0,1—0,2 mol.

The cf-growth of this alcohol is typical in appearance as well as in completeness. The long, thin hyphae hang together in large colonies in which the cells form an airy and porous tissue. The colonies often reach a size that they may be seen macroscopically as cotton-like lumps swimming in the culture fluid. Among all the alcohols studied this is the one which most easily gives cf-growth. Its active zone is unusually

wide, which is connected with the rather high position of the lethality limit.

	Normal primary	Normal secondary	Iso	Tertiary
Tested zone:	0,01—2	0,01—0,5	0,01—2	0,01—2 mol
Lethality:	0,2 —2	0,5	0,2 —2	1 —2 »
Toxicity:	0,05—0,1	No	0,05—0,1	0,1 —0,5 »
Cf-reaction:	0,01—0,1	0,1 —0,2	0,02—0,1	0,05—0,5 »

## 5. PENTANOL, $C_5H_{11}OH$ .

a. *Iso-pentanol* (<sup>29</sup>/<sub>1</sub>, 44). — Concentrations: 0,005—0,5 mol. Above 0,1 mol lethality. Cf-growth occurs in (0,005)—0,01—0,05 mol. Numerous but little developed cf-forms were found in 0,05 mol after 20 hours. At the same time 0,02 mol had preponderantly normal cells; only 10 % cf-forms were seen. This concentration was the highest one giving full fermentation in 2 days. 0,05 mol did not get into fermentation until after 17 days. After 2 days 0,02 mol contained many complicated cf-colonies. These fell apart later on, so after 5 days only few-celled cf-forms were met with, and after 17 days most of the cells were normal.

The cf-forms of pentanol were characteristic. In the strongest concentration, 0,05 mol, they were little developed (Fig. 3: 43—49). They consisted of one rounded cell with one to three narrow tubes. Sometimes the tube made an effort to round itself off into a more normal bud cell, often evidently without success. It then continued growing out into a thin tube, the proximal, basic part of which showed a spherical swelling (Fig. 3: 47, 48). In lower concentrations (0,02 mol) a special type of cf-forms occurred having broad, somewhat indented cells with irregular outline (Fig. 3: 50—52). Certain cells of these cf-forms could form wide, curved tubes with small irregular humps, the whole cell being reminiscent of a maple seed. This type occurred in two separate experiments with iso-pentanol, but has scarcely been observed elsewhere, so it may be characteristic of this alcohol.

b. *Tertiary pentanol* [*Amylene hydrate*,  $(CH_3)_2 \cdot C(OH) \cdot CH_2 \cdot CH_3$ ] (<sup>11</sup>/<sub>8</sub>, 43). — Only very preliminary results are available from this substance. After a test in a moist chamber resulting in positive cf-reaction one series of concentrations between 0,001 and 2 mol was arranged. After 20 hours 0,5—2 mol had killed the cells, 0,1—0,2 mol showed cf-growth, 0,2 mol in a high degree (87 %).

	Iso	Tertiary
Tested zone:	0,005—0,5	0,001—2 mol
Lethality:	0,1 —0,5	0,5 —2 »
Toxicity:	0,02 —0,05	0,2 —0,5 »
Cf-reaction:	0,01 —0,05	(0,1)—0,2 —0,5 »

6. OCTANOL,  $C_8H_{17}OH$ .

a. *Orientating experiment* ( $^{80}/_9$ , 43). — Concentrations: 0,001—0,2 mol. 0,05—0,2 mol had a killing action. In 0,02 there occurred little development, but the cells showed clear cf-tendencies before dying. Typical cf-forms were found in 0,005—0,01 mol. Even in 0,002 clear cf-tendencies were perceivable after 24 hours.

b. *New series* ( $^{30}/_1$ , 44). — Concentrations: 0,001—0,05 mol. The results were in agreement with the preceding series, but in this series 0,005 mol was almost lethal, cf-forms being found only in 0,002 mol and single ones in 0,001 mol. The cf-forms are less developed in this alcohol than in the former ones. Usually they consist only of one cell with an outgrowing tube, larger colonies not being formed. It is evident that the threshold of cf-growth lies rather close to the toxicity threshold, which has the result that any decided cf-growth can occur only in one concentration step without almost total lethality.

Tested zone:	0,001—0,05 mol
Lethality:	(0,005)—0,01—0,05 mol
Toxicity:	0,002—0,005 mol
Cf-reaction:	0,002—0,005—(0,01) mol.

7. LAURYL ALCOHOL (DODECANOL,  $C_{12}H_{25}OH$ ).

Of this as of the following alcohols only qualitative tests were made with saturated solutions. Five cultures were put within the saturated zone with different excess of undissolved substance. The quantity of substance in these cultures corresponded to 0,001—0,01 mol. In none of these tests did total lethality occur. The often encountered phenomenon that the lethality percentage increases with increasing excess of precipitate in saturated solution is very clear:

Excess corresponding to a concentration of:	0,001	0,002	0,01 mol
Lethality %	38,7	84,4	88,2
Fermentation after 2 days	++	+	—

In none of the cultures were any typical cf-forms found, although a general tendency to elongated cell-growth was common.

## 8. HIGHER ALCOHOLS.

a. *Cetyl alcohol* (*Hexadecanol*,  $C_{16}H_{33}OH$ ). — Only one solution was tested, viz. saturated solution with excess of precipitate. This proved perfectly harmless to the yeast cells, which showed a lethality of only 6 % and entered into full fermentation in 2 days. The tendencies to cf-growth were somewhat more definite than in the preceding. No more developed cf-colonies were found, however, although simple involution forms were common.

b. *Octadecanol*,  $C_{18}H_{37}OH$ . — One saturated solution with excess of precipitate was tested. Lethality: 2,2 %, full fermentation in 2 days. Like the preceding one it showed a certain tendency to cf-growth, although less pronounced than this.

## 9. SURVEY OF THE ALIPHATIC ALCOHOLS.

Certain interesting points are apparent on surveying the experimental results of the different alcohols investigated. As is well familiar from many experiments and is stated in the so-called rule of the homologous series of RICHARDSON, the activity of the alcohols increases with increasing number of C-atoms. This has been pointed out for the alcohols several times, for instance, by SEGAL (l. c.) concerning the alcohols tested by him. Even more than the number of C-atoms, however, the water solubility of the alcohols gives a picture of their toxicity (RICHET's rule). Lethal concentrations of the different alcohols met with in the present experiments may be roughly correlated with the solubilities in the following manner:

Solubility	Lowest lethal concentration	Number of alcohols tested
$\infty$	1	5
1	0,2	3
0,3	0,1	1
0,01	0,005	1
< 0,001	no lethality	3

The lethality threshold sinks up to a solubility of 0,01. Below that there appears the well-known »cut-off» in toxicity, which depends on the lethality threshold lying above the solubility; concentrations strong enough to act lethally cannot be attained (cf. FERGUSON, 1939).

Another measure of the toxicity may be had from an estimation of the time period necessary for a culture to reach full fermentation. The control cultures, normally, were fermenting after 2 days. Below

are given the highest concentrations of each treatment allowing full fermentation in 2 days; in these cultures consequently no significant toxicity can be present:

Methanol . . . . .	1	mol
Ethanol . . . . .	1	»
Iso-propanol . . . . .	0,5	»
Normal propanol . . . . .	0,2	»
Tertiary butanol . . . . .	0,2	»
Normal and iso-butanol . . . . .	0,05	»
Iso-pentanol . . . . .	0,02	»
Octanol . . . . .	0,002	»
Lauryl, cetyl and octadecyl alcohol . . . . .	Saturated solution	

This estimation of the fermentation is, of course, rather inexact. It would have been preferable to determine the fermentation intensity by quantitative methods. Exact cell counts for determining the rapidity of growth of the cultures were undertaken and might have been used for measuring the toxicity. Since the present experiments are largely of an explorative nature, however, I prefer not to press the results too hard. For the present a rough estimate of the main lines seems to be sufficient. In determining the toxic zones in the preceding paragraphs, the decrease in cell number in comparison with the controls was taken into account for each tested substance.

The main interest of this investigation has been directed to the cf-reaction. In Table 1 a record has been made of the concentrations of the different alcohols which have given any certain frequency of cf-forms after 20 hours. It is seen that all alcohols have shown at least tendencies to cf-growth. They certainly all possess activity, though their efficiency is highly variable (the terms »activity» and »efficiency» are used in the same meaning as in ÖSTERGREN, 1944, pp. 434—435). The correlation between cf-activity and water solubility is evident.

The efficiency of a substance for inducing a certain reaction may be judged in different ways. The effective concentration zone, measured as the number of concentrations in a certain dilution series which have given the reaction, is one measure. The maximal frequency of cf-forms attained in a substance is another. The number of cells present in the individual cf-colonies is a third measure. These measures may be found in Table 1, and it is seen that they are correlated to each other. Not taking into account the different isomeres of each alcohol, the following is found:



Alcohol	Number of concentrations with cf-forms	Maximal percentage of cf-forms	Average cell number in each cf-colony
Methanol . . . . .	1	60,7	4,5
Ethanol . . . . .	1,5	60,0	3,4
Propanol . . . . .	2	92,9	3,7
Butanol . . . . .	3,7	100,0	5,8
Pentanol . . . . .	4	100,0	4,4
Octanol . . . . .	2	4,3	3,5

A fourth criterion of the efficiency of a substance may be had by studying the cell-shape within the cf-colonies. Cf-colonies of long hyphae with parallel or irregular outlines must be considered more deeply narcotized than such as have undulated walls or such as have tubes alternating with normal cells.

It will be seen that all these criteria point to a maximum of efficiency at 4 to 5 C-atoms: 4 concentrations give effect, 100 % of the cells may turn into cf-forms, the average number of cells in single cf-colonies was about 5, the cell-shape of the cf-forms in butanol and pentanol is extremely elongated. Tertiary butanol even exceeds camphor in this respect.

#### IV. OTHER ALIPHATIC SUBSTANCES.

##### 1. ETHYL ETHER.

a. *Orientating experiment* ( $\frac{8}{10}$ , 43). — Concentrations: 0,01—2 mol. The concentrations, however, are probably somewhat inexact, since the experiment was performed in test tubes closed with corks. The evaporation of the ether may not have been effectively checked. Killing concentrations: 1—2 mol; cf-growth occurred in 0,1—0,5 mol, in 0,5 almost to 100 %.

b. *New series* ( $\frac{2}{10}$ , 44). — Concentrations: 2, 3, 4, 5, 6, 7 volume % (about 0,2—0,7 mol). This experiment was made in soda water bottles with air-proof stoppers. All except 2 % showed a high degree of lethality after 20 hours, 2 % had a lethality of 48 %. This concentration started to ferment weakly after 2 days. After 6 days 4—7 % were all dead, 3 % showed no development but contained living cells, 2 % had come into lively fermentation.

Cf-forms were found occasionally in the lethal concentrations, a certain cf-growth having started before death. Only 2 % showed any

high degree of cf-growth: after 1 and 2 days it held almost 100 % cf-forms. The type of cf-forms (Fig. 4 *a—f*) was consistent in both experiments. Both long hyphae and large bottle-shaped or vesicular cells were formed. These latter may swell up into a considerable volume (Fig. 4 *d, f*), exceeding that of the cf-cells of the aliphatic alcohols.

Tested zone: 0,01—2 mol  
 Lethality: 0,4 —2 »  
 Toxicity: 0,2—0,4 »  
 Cf-reaction: 0,1—0,3 »

## 2. ACETONE.

a. *Orientating experiment* (<sup>8</sup>/<sub>9</sub>, 43). — Concentrations: 0,01—2 mol. Lethality: 2 mol. In 1 mol the development is checked, but after 2 days living cells are still found. Cf-forms are found in 0,2—0,5 mol. In 0,1 mol tendencies to cf-growth are found, but the cells are preponderantly normal. After 2 days large and complicated cf-forms were found in 0,5 mol.

b. *New series* (<sup>2</sup>/<sub>2</sub>, 44). — Concentrations: 1, 2, 3, 4, 5 volume % (0,14—0,68 mol). 5 % showed no development, 4 % gave weak fermentation after 5 days and then contained quite large cf-forms, 3 % gave little development in 2 days, most cells showing cf-growth. In five days this concentration was in full fermentation, and it then contained mostly normal cells. 1 and 2 % fermented already after 2 days. These two contained 2,7 % and 58,7 % cf-forms respectively after 20 hours.

The cf-forms of acetone have a characteristic type (Fig. 4 *g—n*) resembling the cf-forms of ether. Quite long tubes are formed, which often begin to swell up into large pumpkin- or pear-like formations. These large cells may often take an irregular, indented outline (Fig. 4 *m*) directly indicating lacking growth control. The largest cells often take especially strong staining in aceto-carmin, which may point to sublethality.

Tested zone: 0,01—2 mol  
 Lethality: 2 »  
 Toxicity: 0,4 —1 »  
 Cf-reaction: 0,2 —0,5 »

## 3. CHLOROFORM.

a. *Orientating experiment* (<sup>8</sup>/<sub>9</sub>, 43). — Concentrations: 0,0005—0,1 mol. Lethality: 0,05—0,1. 0,05 gave some tendencies to cf-growth before dying. 0,02 gave a considerable frequency of cf-forms after 20

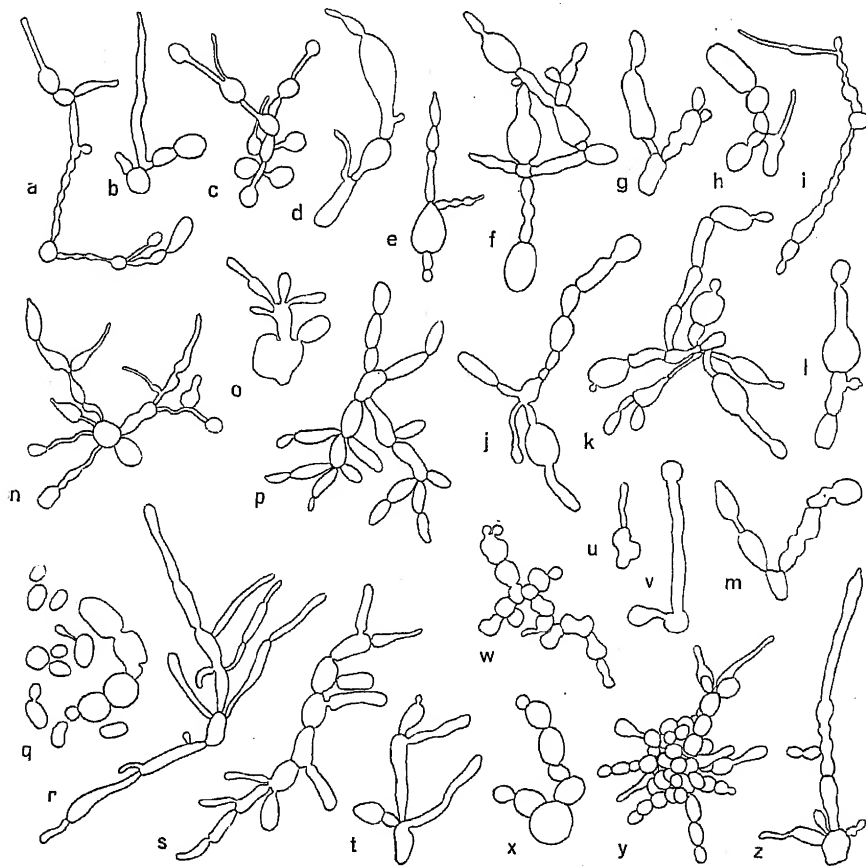


Fig. 4. Outline drawings of cf-forms induced by various aliphatic substances: ethyl ether (a—f), acetone (g—n), chloroform (o, p), paraldehyde (q, w), chloral hydrate (r—t), urethane (u—v, x—z). —  $\times 500$ .

hours, but after 2 days normal cells preponderated. Traces of cf-growth were seen in 0,005—0,01 mol.

b. *New series* (<sup>2</sup>/<sub>2</sub>, 44). — Concentrations: 0,05, 0,10, 0,15, 0,20, 0,25, 0,30 volume % (0,006—0,04 mol). After 20 hours 0,20—0,30 % was dead, as were the majority of cells in 0,10—0,15 %. 0,05 % showed a lethality of 52 % and a rather high proportion of cf-forms. This concentration

began to ferment weakly after 2 days and reached full fermentation in 5 days. None of the stronger concentrations were then yet fermenting.

The cf-forms (Fig. 4 o, p) are initiated as tubes, but often develop into a more rounded form. The cells may acquire a rather normal shape but are larger than normally. The average type of cf-form is less striking than in ether and acetone. Chloroform is less efficient as cf-substance, since it acts toxically in those concentrations which give the reaction. This may also be the cause of the less extreme form of the cf-cells.

Tested zone: 0,0005—0,1 mol

Lethality: 0,02,1—0,1 »

Toxicity: 0,006 —0,01 »

Cf-reaction: 0,005 —0,01 »

#### 4. PARALDEHYDE.

a. *Orientating experiment* (<sup>8</sup>/<sub>9</sub>, 43). — Concentrations: 0,002—0,5 mol; lethality: 0,2—0,5 mol; cf-reaction: 0,05 (only solitary cf-forms) and 0,1.

b. *New series* (<sup>2</sup>/<sub>2</sub>, 45). — Concentrations: 0,5, 1,0, 1,5, 2,0 volume % (0,04—0,15 mol). None of these concentrations was lethal. 2 % was the only one which did not get into fermentation in 2 days. It was in weak fermentation, however, after 5 days. Cf-forms were found in 0,5—1,5 %, although rather infrequently.

The cf-forms are not very advanced (Fig. 4 q); often they consist only of one large cell and one outgrowing tube, often of the same breadth as the mother cell. A rather high frequency of giant cells with normal cell-form were found.

Tested zone: 0,002—0,5 mol

Lethality: 0,2 —0,5 »

Toxicity: 0,1 —0,15 »

Cf-reaction: (0,05)—0,1—0,15 mol.

#### 5. CHLORAL HYDRATE.

a. *Orientating experiment* (<sup>8</sup>/<sub>9</sub>, 43). — Concentrations: 0,001—0,2 mol. Lethality: 0,2 mol. Also 0,1 showed no development in 2 days, but the cells were not dead. 0,02—0,05 mol had a high frequency of cf-forms after 20 hours, 0,01 had mostly normal cells although with clear cf-tendencies.

b. *Two complementary concentrations* ( $\frac{1}{2}$ , 44). — 0,04 and 0,06 mol. Both showed high frequency of cf-forms. The general development of the cultures was slow, 0,04 mol fermented after 3 days.

The cf-forms, easily produced in several concentrations of this substance, were often of a rather extreme type (Fig. 4 *r-t*). Long hyphae grow out and are retained as cells, from which new lateral hyphae are sent out. A certain regularity in the structure of the cf-colonies was thus acquired, as already discussed above in the general chapter.

Tested zone: 0,001—0,2 mol  
 Lethality: 0,2 »  
 Toxicity: 0,02—0,1 »  
 Cf-reaction: (0,01)—0,02—0,1 mol.

## 6. URETHANE.

a. *Orientating experiment* ( $\frac{5}{3}$ , 43). — Concentrations: 0,01—2 mol. Lethality: 1—2 mol. 0,5 gave no development, some living cells remained after 2 days. In 0,2 mol there occurred plenty of cf-forms after 1 and 2 days, 0,1 mol had cf-forms only after 1 day, after 2 days the cells were again normal.

The cf-forms have an interesting type (Fig. 4 *u-v*, *x-z*). Very long hypha-tubes may, it is true, be found now and then, but these usually later on give rise to many small more or less rounded cells. After 2 days in 0,2 mol, for instance, numerous cf-colonies of 20 to 40 cells were found, all cells in the interior of the colonies being spherical (Fig. 4 *y*). They hang together even after heavy shaking and may be compared with the cf-colonies after long treatment with ethanol (Fig. 3: 18). After some time the colonies tend to fall apart, and it is then seen that the size of the cells is very varying and that their outlines are often quite irregular.

Tested zone: 0,01—2 mol  
 Lethality: 1 —2 »  
 Toxicity: 0,2 —0,5 »  
 Cf-reaction: 0,1 —0,2 »

## 7. VERONAL, ADALINE AND SULFONAL.

These three substances — veronal (diethylbarbituric acid), adaline (*a*-bromo-*a*-ethyl-butyl-urea) and sulfonal (diethyl-sulfon-di-methyl-

urethane) — resemble each other in that saturated solutions do not give lethality and only little poison effect. Their cf-reaction is not very pronounced. In the strongest concentrations small groups of cells occur. The cells of these groups are often somewhat elongated in shape and are often larger than normally. In veronal I found a low percentage of developed cf-forms with distorted tube-like cells.

	Veronal	Adaline	Sulfonal
Tested zone:	0,0005—0,08	0,00005—0,01	0,0001—0,02 mol
Cf-reaction:	0,08	0,01	0,02 »

In these concentrations the cf-reaction is only partial, the majority of the cells being normal.

#### 8. A FEW OTHER SUBSTANCES.

Some data may be given for a few substances in which only preliminary and incomplete experiments have been made. Trichlorethylene and acethylene tetrachloride have been studied in the zone 0,003—0,03 mol. The latter substance gave cf-forms in 0,003 mol, which was the only concentration in the series permitting any development. Two halogen-substituted alcohols were studied, both used in practice as soporifics, viz. avertin (tribromoethyl alcohol) and chloretone (trichloro-*tert*-butylalcohol). Both gave typical cf-reaction in some concentrations.

	Avertin	Chloretone
Tested zone:	0,001—0,1	0,01—0,08
Lethality:	0,02 —0,1	0,03—0,08
Toxicity:	0,01	0,02
Cf-reaction:	0,002—0,005	0,01—0,02

#### 9. SURVEY OF THE ALIPHATIC SUBSTANCES OTHER THAN THE ALCOHOLS.

All substances studied gave at least tendencies to cf-reaction in some concentration. The correlation between activity and water solubility, pointed out concerning the alcohols, is met with also here. The lethality, for instance, behaves in the following manner:

Substance	Solubility	Lowest lethal concentration
Acetone .....	$\infty$	2,0
Urethane .....	11,25	1,0
Ether, chloral hydrate, paraldehyde ..	$\pm 1$	0,2—0,4
Chloroform .....	0,08	0,02

TABLE 2. *The cf-reaction of some aliphatic substances.*

Substance	Molar concentration	Number of cells per cf-colony															Total number of colonies	Average number of cells per cf-form	Percentage of cf-forms	Lethality percentage	Number of days to full fermentation
		1	2	3	4	5	6	7	8	9	10	12	15								
Ether	0,19	12	6	15	3	—	—	—	—	—	—	—	—	36	2,3	90,0	48,1	5			
Acetone	0,41	15	9	6	1	2	1	—	—	—	—	—	—	34	2,1	89,7	47,3	5			
	0,27	2	3	1	8	6	3	3	—	—	—	1	—	27	4,7	58,7	30,3	2			
	0,14	1	1	2	2	—	2	—	1	—	—	—	—	9	4,1	2,7	7,5	2			
Chloroform	0,0063	3	2	3	4	5	2	1	—	—	—	—	—	20	3,8	80,0	51,9	5			
Paradelhyde	0,11	1	7	4	—	1	2	—	—	—	—	—	—	15	2,9	25,9	28,4	5			
	0,08	1	2	2	2	1	7	4	3	1	1	1	—	25	6,0	8,0	7,9	2			
	0,04	—	1	2	—	1	—	1	—	—	—	—	—	5	4,0	1,0	4,4	2			
Chloral hydrate	0,02	—	1	5	6	1	—	2	—	—	—	—	—	15	4,0	51,7	23,7	2			
	0,01	1	3	1	4	3	2	—	1	—	—	—	—	15	4,1	30,0	19,4	2			
	0,005	1	3	3	1	—	—	—	1	—	—	—	—	9	3,1	15,3	15,7	2			
	0,002	1	—	—	3	3	—	—	—	—	—	—	—	7	4,0	13,7	20,3	2			
	0,001	—	—	4	3	1	—	—	—	—	—	—	—	8	3,5	17,8	21,1	2			
Urethane	0,2	—	1	1	2	3	not counted								7	4,0	63,6	38,9	5		
	0,1																—	—	57,7	4,6	2

The following concentrations of the various substances were in fermentation after 2 days and may thus be regarded as the upper limit of non-toxicity:

Acetone .....	0,27 mol
Urethane .....	0,10 »
Chloral hydrate .....	0,02 »
Ether .....	0,15 »
Paraldehyde .....	0,08 »
Chloroform .....	0,005 »

Broadly speaking, a clear negative correlation is valid also here between toxicity and water solubility. No close correlation can be expected in a group of such chemical heterogeneity. It is quite conceivable that, for instance, chloral hydrate shows a specific toxicity, falling outside the unspecific activity, which is correlated to the physical properties of the substances.

Table 2 gives a similar survey of the cf-reaction as was given in Table 1 for the alcohols. Those concentrations of the substances which have induced more than casual cf-forms have been recorded together with some data in connection with the reaction. In chloral hydrate the three lowest concentrations of Table 2 are taken from an experiment not recorded above. They refer to cultures grown at room temperature and are not quite comparable to the other values.

Substance	Active concentration	Maximal frequency of cf-forms	Average number of cells per cf-colony
Ether .....	0,19	90,0	2,3
Acetone .....	0,14—0,41	89,7	3,6
Chloroform .....	0,0063	80,0	3,8
Paraldehyd .....	0,04—0,11	25,9	4,3
Chloral hydrate ..	(0,001)—0,01—0,02	51,7	3,7
Urethane .....	0,1—0,2	63,6	4,0

The activity zones arranged according to water solubility:

Solubility	Activity zone
$\infty$	0,14 — 0,41
11	0,1 — 0,2
$\pm 1$	0,001—0,02; 0,04—0,11; 0,19
0,08	0,0063

A certain correlation between water solubility and activity is evident. Chloral hydrate also shows in the cf-reaction somewhat higher activity than its water solubility would indicate. As expected, the correlation is less distinct in this group than in the preceding group of substances.

## V. ALICYCLIC SUBSTANCES.

### 1. CYCLOHEXANE AND BROMOCYCLOHEXANE.

Only one preliminary experiment was made with these two substances. Concentrations: 0,0001—0,005 mol. No concentration gave complete cf-reaction, but in 0,005 mol of cyclohexane and in 0,002 mol of bromocyclohexane colonies of rounded and irregularly shaped cells occurred.

### 2. CAMPHOR.

Camphor was the first substance in which the reaction was closely studied. It is still one of the most efficient cf-substances, especially when it comes to the induction of multicellular cf-colonies with long, tube-like cells. LEVAN and SANDWALL (1943) found activity in borneol also. Since it seemed of special interest to study substances related to camphor, several experiments were arranged in August, 1943, with various terpene derivatives.

Camphor was studied in the following concentrations: 0,000001—0,01 mol. A detailed testing was made of the zone 0,001—0,01. As already reported by LEVAN and SANDWALL (l. c.), almost complete lethality goes down to 0,008 mol. Below this concentration the cf-reaction reaches close to 100 %. A high degree of reaction is obtained down to 0,003 mol, below this concentration single cf-forms may be found as far down as 0,00001 mol. The lower threshold is thus rather indistinct. The frequency curve of cf-forms rises rapidly, however, at 0,002—0,003 mol (Fig. 5).

It is obvious that the cf-reaction is readily induced by camphor. It occurs in a large interval of concentrations and reaches high frequency in rather intoxic concentrations as well. The cf-cells may be very long tubes, and the colonies may reach a size which is visible macroscopically.

Tested zone:	0,000001—0,01	mol
Lethality:	0,008	—0,01 »
Toxicity:	0,005	—0,007 »
Cf-reaction:	0,002	—0,007 »

## 3. BORNEOL.

While camphor is a ketone, borneol is the corresponding alcohol. It is also efficient in giving the cf-reaction. Its lethal action goes somewhat lower down than that of camphor. Its zone of cf-activity is somewhat narrower. Cf-forms are found between 0,001 and 0,0045 mol, in high frequency only in the zone 0,0035—0,0045. It never reaches the completeness of the reaction induced by camphor.

Tested zone: 0,000001—0,01 mol  
 Lethality: 0,006 —0,01 »  
 Toxicity: 0,002 —0,0055 »  
 Cf-reaction: 0,001 —0,0045 »

## 4. BORNYL ACETATE AND BORNYL CHLORIDE.

These two esters of borneol are both inactive as far as the cf-reaction is concerned. They are decidedly more toxic than the preceding substances of this chapter.

	Acetate	Chloride
Tested zone:	0,0002—0,01	0,00001—0,001 mol
Lethality:	0,001 —0,01	0,0002 —0,001 »
Toxicity:	0,0006—0,0008	0,00008 »

## 5. CAMPHOR OXIME.

Here the keto-group ( $>C=O$ ) of camphor is changed to an oxime ( $>C=NOH$ ). It is lethal only in saturated solution plus a considerable precipitate corresponding to a concentration of 0,04 mol or higher. It has activity in a rather wide zone, but always in a low degree. The cf-forms are typical.

Tested zone: 0,00005—0,08 mol  
 Lethality: 0,04 —0,08 »  
 Toxicity: 0,003 —0,04 »  
 Cf-reaction: 0,003 —0,02 »

## 6. CAMPHORIC ACID.

This is a two-basic acid and it is the easiest soluble one of the camphor derivatives here tested. It gives lethality in 0,08 mol. In 0,02—0,04 mol numerous tendencies to cf-forms were met with after one day's treatment. There occurred elongated cells, single tube-like bud cells,

etc. Also after 2 days some cf-forms were seen in these concentrations, their frequency, however, being little.

Tested zone: 0,0001—0,08 mol

Lethality: 0,08 »

Toxicity: 0,01 —0,04 »

Cf-reaction: 0,02 —0,04 »

## 7. CAMPHENE.

This is an unsaturated hydrocarbon, and it is among the least soluble ones of these substances. It gives no cf-reaction.

Tested zone: 0,00004—0,004 mol

Toxicity and lethality: 0,0002 —0,004 »

## 8. TERPINEOL AND MENTHOL.

These substances, one saturated and one unsaturated alcohol, were tested. They gave no cf-reaction, terpineol may give some traces of reaction in 0,001 mol.

	Terpineol	Menthol
Tested zone:	0,000001—0,02	0,000001—0,01 mol
Lethality:	0,008 —0,02	0,002 —0,01 »
Toxicity:	0,002 —0,008	0,0006 —0,002 »

## 9. SURVEY OF THE ALICYCLIC SUBSTANCES.

Within the group of camphor derivatives an evident correlation is found between solubility and activity. It may be pointed out, as has already been done several times, that gross sources of error occur. The undeniable regularity of the solubility correlation indicates, however, that the fundamental principle underlying these conditions has not been obscured by the experimental uncertainty. Sources of error are, first, the difficulty in determining the water solubility of the substances. Unfortunately, for the substances concerned here the current table-works very seldom give other values of solubilities than »very slightly soluble» or »insoluble». I had the possibility of making a rough estimate of the solubility when the series were mixed. The critical values — the weakest concentration with precipitate and the strongest one without precipitate — were controlled both at the preparation of the series and after keeping the solutions in shut bottles for one day. The more important substances were also controlled in solutions of distilled

water. Another source of error is the difficulty of obtaining pure substances. The camphor derivatives tested here were kindly presented by the Royal Technical College of Stockholm, where they had been produced during students' class work.

The camphor derivatives represent a rather wide scale of solubilities, and they are therefore well suited for a testing of the present working hypothesis. Arranged in decreasing solubility they will form the following series:

Substance	Solubility	Lowest lethal concentration	Highest non-toxic conc.
(1) Camphoric acid . . . . .	0,02 —0,04	0,08	0,006
(2) Camphor oxime . . . . .	0,008 —0,009	0,04	0,004
(3) Camphor . . . . .	0,004 —0,005	0,008	0,003
(4) Borneol . . . . .	0,001 —0,002	0,006	0,001
(5) Bornyl acetate . . . . .	0,0008 —0,001	0,001	0,0004
(6) Camphene . . . . .	0,0004 —0,0006	0,0002	0,0001
(7) Bornyl chloride . . . . .	0,00008—0,0002	0,0002	0,00006

As seen, a striking correlation between toxicity and solubility is present.

Only four of the substances have cf-efficiency. It is rather suggestive that these four substances are those of the above series with the highest water solubility (Nos. 1—4). This indicates that the activity of the rest of the substances is cut off by the sinking solubility, as was found in the aliphatic alcohols: the activity line passes above the solubility line. The cf-reaction occurs in the following zones:

Camphoric acid . . . . .	0,02 —0,04	mol (tendencies)
Camphor oxime . . . . .	0,003—0,02	»
Camphor . . . . .	0,002—0,007	»
Borneol . . . . .	0,001—0,0045	»

As already mentioned, camphor and also camphor oxime may give single cf-forms in concentrations decidedly below those recorded above. Thus, in one experiment in 1942 camphor gave, counted as averages of  $1/25$  mm<sup>3</sup>, the following number of cf-forms in weak concentrations:

Concentration . . . . .	0,00001	0,0001	0,0003	0,0007	0,001	mol
Number of cf-forms . . . .	0,8	0,8	1,2	3,6	0,8	»

The concentration 0,002 mol is considered as threshold value, since it is first in this concentration that any considerable frequency of cf-forms begins to appear.

TABLE 3. *The cf-reaction of camphor and related substances.*

Substance	Time of treatment	Molar concentration	Number of cells per cf-form		Total number of cf-forms	Percentage of cf-forms	Lethality percentage	Number of cells in the control	Substance	Time of treatment	Molar concentration	Number of cells per cf-form		Total number of cf-forms	Percentage of cf-forms	Lethality percentage	Number of cells in the control
			Aver-	Maxi-								Aver-	Maxi-				
C a m p h o r	1 day	0,007	3,8	8	156	91,5	38,2	6,3	B o r n e o l	1 day	0,0045	2,6	6	11	33,3	78,2	3,7
		0,0066	3,5	9	25	73,5	27,7	5,9			0,0040	3,1	9	140	22,5	40,3	4,6
		0,006	3,5	9	159	85,0	32,2	4,9			0,0035	3,9	12	130	21,2	17,5	5,4
		0,005	5,0	21	191	34,1	22,2	13,5			0,0030	3,8	12	183	6,6	5,6	24,2
		0,004	3,9	12	113	5,1	2,3	33,3			0,0025	4,3	8	66	1,9	4,8	30,8
	2 days	0,0033	3,2	7	22	6,5	6,6	45,7		2 days	0,0020	4,0	10	27	0,5	4,4	39,9
		0,003	4,7	12	106	1,5	4,7	52,0			0,0010	2,0	5	8	0,5	0,7	60,3
		0,002	4,8	13	51	0,7	2,5	61,3			0,004	5,5	11	10	3,3	50,0	1,1
		0,001	3,8	7	17	0,3	5,3	67,8			0,002	3,1	7	13	0,06	2,3	79,1
C a m p h o r	2 days	0,007	4,4	30	432	18,5	4,9	22,0	C a m p h o r o x i m e	1 day	0,01	4,4	11	52	6,7	1,9	14,2
		0,0066	4,9	20	62	6,1	7,9	17,1			0,009	4,4	16	62	4,5	1,8	25,5
		0,006	4,8	17	264	12,4	6,7	15,1			0,008	5,2	12	41	7,1	9,3	11,6
		0,005	4,2	19	186	4,5	3,4	31,5			0,007	4,6	10	49	4,7	4,6	19,8
		0,004	4,6	17	64	1,3	1,6	51,6			0,005	3,4	7	36	1,5	1,6	43,5
		0,003	3,5	18	91	0,6	2,7	74,7			0,004	2,7	5	35	1,1	1,2	58,7
		0,002	3,9	8	51	0,3	2,1	81,0			0,003	3,1	9	36	0,8	2,5	23,6
		0,001	2,7	5	41	0,1	3,2	116,3									

The cf-reaction of the four substances concerned shows some characteristic features. The cf-reaction induced by camphoric acid is very weak, only tendencies to cf-forms being noticed. Camphor oxime has a wide activity range, 0,003—0,02 mol. The reaction never becomes very predominating, however. In all concentrations normal cell-growth is most common, the frequency of cf-forms never exceeds 10 %. Camphor has a high frequency of cf-forms in a narrow zone, 0,006—0,007 mol. Here the cf-growth is almost 100 %. The frequency curve of

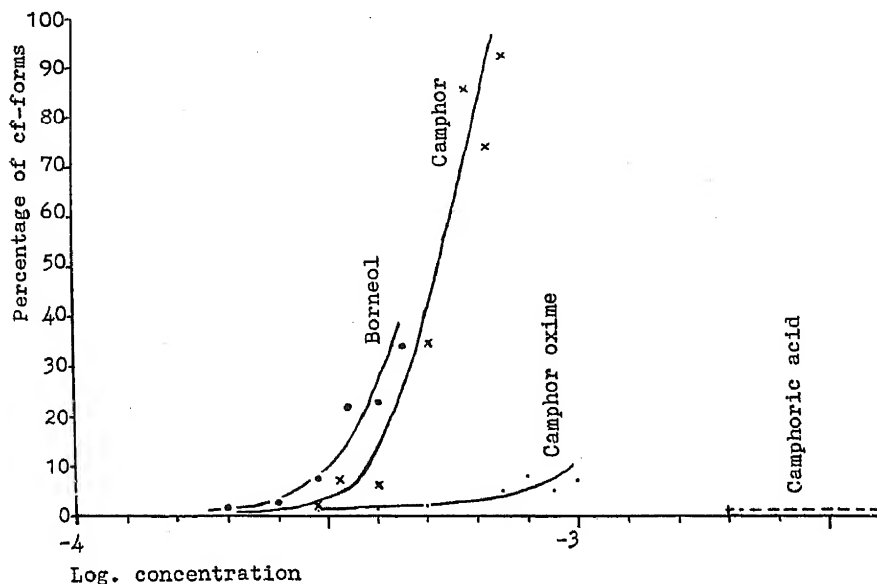


Fig. 5. Graph of the cf-activity of camphor derivatives.

cf-forms in borneol shows a similar steep increase as in camphor. It starts at 0,002 mol but is interrupted by lethality at 0,0045 mol. It has then reached 33 %. These conditions are represented graphically in Fig. 5.

Some results of cell counts in different concentrations of the alicyclic substances are collected in Table 3. Here are recorded the total frequency of cf-forms found in the cultures, the lethality and toxicity, the latter given in relation to the cell number of the controls. In camphor and borneol some data are given from some 2-day treatments as well. A summary of some of this material is already published in LEVAN and SANDWALL (l. c.).

The number of cells within the cf-forms is about the same in the three substances, 2—5 cells on an average. The variations are greater after 2 days, when the older cf-colonies have attained a higher cell number. At the same time the weaker concentrations no longer exercise any strong effect on the yeast, which evidently becomes resistant to the treatment, so here the cf-forms have a tendency to fall apart. This is most probably the cause of the low mean values during the second day in camphor and borneol. Even in the stronger concentrations of camphor there occurs after 2 days a considerably decreased lethality and increased cell number. Simultaneously, the frequency of cf-forms decreases owing to an acquired resistance towards the treatment.

It is clearly seen from Fig. 5 that among the substances tested here camphor represents optimum for the induction of the cf-reaction. It has a high frequency of cf-forms in such concentrations as show but little lethality.

## VI. AROMATIC SUBSTANCES.

### 1. BENZENE.

Only explorative experiments have been made with benzene as with other aromatic substances tested. Most of the experiments with aromatic substances were made in August, 1943; some control series were made on later occasions too. Of benzene, concentrations from 0,004 to 0,016 mol were tested. The stronger solutions 0,013—0,016 were totally lethal, 0,004 mol had no perceptible effect, 0,008—0,01 mol had rather strong toxic effect. In the latter concentrations there occurred a varying frequency of small cell colonies of 4—8 cells without any striking deviation in cell-forms. This is the typical cf-reaction of aromatic monocyclic substances.

One benzene series laid down in July, 1946, contained the concentrations 0,007, 0,009, 0,010 and 0,012 mol. Especially 0,009 and 0,010 mol showed high frequency of cf-forms, while 0,012 mol showed little development in 1 day. Later on this highest concentration developed numerous cf-forms. Fig. 2 is drawn from cells treated 16 days in this concentration.

The cf-forms of benzene as of most aromatic substances do not have the elongated tube-like shape typical of the cf-forms induced by most aliphatic substances. The cells are rounded or have a more irregular form. The colonies never actually attain the size or cell number

of, for instance, camphor, which may depend on the active concentrations of the aromatic substances often being rather toxic.

Tested zone: 0,004—0,016 mol  
 Lethality: 0,013—0,016 »  
 Toxicity: 0,008—0,012 »  
 Cf-reaction: 0,008—0,012 »

## 2. BENZENE HOMOLOGUES.

In this series toluene, meta-xylene and mesitylene were studied. All of them gave cf-forms of the benzene-type. The cf-reaction of toluene was rather unclear.

	Toluene	Xylene	Mesitylene
Tested zone:	0,002—0,004	0,0008—0,0016	0,00018—0,00056 mol
Lethality:	0,0032—0,004	0,0013—0,0016	0,00016—0,00056 »
Toxicity:	0,0024—0,0028	0,0011	0,00035—0,00042 »
Cf-reaction:	0,0024	0,0010—0,0011	0,00035—0,00039 »

## 3. NITROBENZENE.

Only two concentrations were tested, 0,01 and 0,02 mol. The latter was lethal, the former had strong toxic effect and contained numerous typical cf-forms of the benzene-type.

## 4. ANILINE.

Six concentrations between 0,04 and 0,4 mol were tested. 0,08—0,4 mol had no development and probably had a lethal action. Only 0,04 mol showed some growth together with decided toxicity. This concentration gave almost 100 % cf-reaction.

## 5. PHENYL URETHANE.

This substance gave typical cf-forms, also sometimes with quite elongated cf-cells.

Tested zone: 0,002—0,02 mol  
 Lethality: 0,02 »  
 Toxicity: 0,002—0,016 »  
 Cf-reaction: 0,012—0,016 »

## 6. ACETOPHENONE.

This substance gave only partial cf-reaction in my experiments.

Tested zone: 0,005—0,05 mol  
 Lethality: 0,01 —0,05 »  
 Toxicity: 0,01 —0,03 »  
 Cf-reaction: 0,01 »

## 7. AROMATIC ALCOHOLS.

Two phenyl ethyl alcohols were tested, primary and secondary. The latter gave a clear cf-reaction, the former gave only tendencies.

	Primary	Secondary
Tested zone:	0,001—0,2	0,001—0,2 mol
Lethality:	0,02 —0,2	0,05 —0,2 »
Toxicity:	0,01	0,02 »
Cf-reaction:	(0,005—0,01)	0,01—0,02 »

Thymol was also tested, and the related hydrocarbon cymene. Neither of them gave any cf-reaction.

	Thymol	Cymene
Tested zone:	0,00001—0,01	0,00001—0,01 mol
Lethality:	0,002 —0,01	0,0005 —0,01 »
Toxicity:	0,0005 —0,001	0,0002 »

## 8. SURVEY OF THE AROMATIC SUBSTANCES.

Although the aromatic substances have given rather meagre results concerning the main subject of study, the cf-reaction, they have considerable interest for the general conclusions of the present work. They include substances with very varying water solubility and therefore give several good instances of the solubility-activity correlation.

It is of interest to study such a group of related substances as benzene and its methylated derivatives. These form a series of decreasing solubility from benzene with a solubility of 0,013—0,016 mol, determined in wort, to mesitylene, the solubility of which is some 40 times lower, 0,0004—0,0005 mol, determined and kindly communicated by ÖSTERGREN. The relation between water solubility, on one side, and lethality, toxicity and cf-reaction on the other, is evident:

Substance	Lowest lethal concentration	Highest non-toxic concentration	Cf-reaction
Benzene . . . . .	0,013	0,004	0,008 — 0,01 mol
Toluene . . . . .	0,0032	0,002	0,0024 »
Xylene . . . . .	0,0013	0,001	0,001 — 0,0011 »
Mesitylene . . . . .	0,00046	0,0002	0,00035 — 0,00039 »

If the eleven tested aromatic substances are divided into four groups according to their water solubility, the correlation is also evident:

Group	Substance	Solubility	Lowest lethal concentration	Highest non-toxic concentration	Cf-reaction
1	Aniline	0,1 — 1,0	0,08	0,04	0,04
2	Phenyl ethyl alcohol	0,01 — 0,1	0,01 — 0,05	0,002 — 0,005	0,005 — 0,02
	Acetophenone				
	Nitrobenzene				
	Benzene				
3	Phenyl urethane	0,001 — 0,01	0,001 — 0,01	0,0002 — 0,002	0,001 — 0,0024
	Thymol				
	Toluene				
4	Xylene	0,0001 — 0,001	0,0004 — 0,0005	0,0001 — 0,0002	0,00035 — 0,00039
	Mesitylene				
	Cymene				

The agreement is actually greater than is seen from the above schematic table. If the substances are arranged in detail according to falling solubility, they are at the same time arranged according to falling lethality, falling toxicity and falling cf-threshold. In thymol the toxicity goes a few concentrations further down than expected from its water solubility. I do not wish, however, owing to the inaccuracy with which the water solubilities have been determined, to draw too far-reaching conclusions. The general tendency of the material is consistent, even if exceptions often occur in the details.

## VII. MODIFICATIVE INFLUENCES ON THE CF-REACTION.

All treatments described in the preceding chapters have been made on yeast grown under optimal conditions. At the time of inoculation the yeast has been in vigorous fermentation, and immediately before it has been inoculated several times over on fresh wort. It has for a long period had good nutrition conditions. The experiments have been made at a constant temperature of 25 centigrades.

Now it was early noticed that even small changes in the environ-

ment could bring about considerable variations in the results of the experiments. Thus, some explorative experiments were made in Svalöf at room temperature (17—20°) without using a thermostat. It was found that cf-forms were obtained much more easily under these conditions than at 25°. Similarly it turned out to be hard to obtain cf-forms in ethanol if another yeast than »fuzzy heads» was employed. In the following pages a brief report will be given of three types of experiments which throw some light upon the significance of the general condition of the yeast, the influence of the temperature and, finally, the induction of a certain resistance of the yeast towards toxicity and cf-reaction.

### 1. THE PHYSIOLOGICAL CONDITION OF THE YEAST.

In these experiments three different types of bottom yeast were used, all belonging to the same clone as that of the above experiments:

- A. »Fuzzy heads» (= hochkräus yeast),
- B. Seed yeast from fermenting vessel,
- C. Bottoms (cask deposit, »geläger» yeast).

A was taken from fermenting vessels during practical beer brewing at vigorous fermentation, B had sedimented and was taken immediately after finished fermentation, C had been stored for a couple of months in storage tanks.

The experiments were arranged in the following way: Series of test-tubes with 10 cc of wort containing different concentrations of the substances under experiment were simultaneously inoculated with approximately the same cell number of A, B and C. The course of the fermentation was followed daily. On various occasions samples were taken for microscopical control. Whereas the results concerning general toxicity were conclusive, I dare not yet deal with differences in cf-reaction.

a. *Methanol* ( $^{27}/_{10}$ , 45). — Concentrations: 5, 6, 7, 8, 9, 10, 11 volume % (1,2—2,7 mol). From earlier experiments it was known that 2 mol (about 8 %) does not come into full fermentation until after 17 days, i. e. a very strong toxic effect.

In A (fuzzy heads) no fermentation at all started during the first two days. On the 3rd day 5 and 6 % started, on the 4th day also 7 % and on the 6th day 8 % started, in the last case, a very weak fermentation. 9—11 % had no development at all. The only culture which had completed fermentation on the 6th day was 5 %.

The B series showed a striking difference: 5 and 6 % already started fermentation on the 1st day, and on the 2nd day all concentrations 5—11 % were in fermentation. The fermentation proceeded rapidly, so that on the 6th day all cultures had finished except 9—11 %, which were then in full fermentation. Yeast B (recently sedimented yeast) thus showed an immensely greater tolerance towards methanol than yeast A.

The C series (cask deposit) took an intermediary position. No fermentation occurred on the 1st day, but on the 2nd day 5—9 % started, and on the 6th day 5, 6 and 7 % had finished fermentation, 8—10 % were in full fermentation and 11 % had only just started.

b. *Ethanol* ( $^{20}/_6$  and  $^{27}/_6$ , 45). — Two series were made both with the following concentrations: 5, 6, 7, 8, 9 and 10 volume % (0.9—1.7 mol). Earlier experiments had shown that 10 % did not arrive into fermentation in 17 days, while in this time 9 % showed a weak start of the fermentation.

The A series showed, as expected, similar behaviour as that just mentioned: No concentration started fermenting on the 1st day, 5—7 % started on the 2nd day, 8 % on the 4th, and on the 6th day even 9 % fermented although not so strongly as the four weaker concentrations. 10 % did not come into fermentation.

Within the B series fermentation started for 5—7 % already on the 1st day, and on the 2nd day all concentrations were in fermentation, the concentration 10 %, however, but hesitatingly.

The C cultures did not show fermentation until the 2nd day (5—8 %), on the 4th day 9 % started, 10 % did not get into fermentation. These differences are more clearly seen from the following table, which includes estimates of the cell numbers of methanol and ethanol. The estimates were made after one day's treatment:

%	A		B		C	
	Methanol	Ethanol	Methanol	Ethanol	Methanol	Ethanol
5 ....	0	1	10	10	2	4
6 ....	0	1	6	8	2	3
7 ....	0	1	3	8	2	1
8 ....	0	0	2	7	1	1
9 ....	0	0	2	2	2	0
10 ....	0	0	3	3	1	0
11 ....	0	—	3	—	0	—
0 (control)	10	10	10	10	10	10

c. *Tertiary butanol* ( $^{27}/_6$ , 45). — Concentrations: 0,5, 1,0, 1,5, 2,0, 2,5, 3,0 volume % (0,05—0,32 mol). It was known that 0,2 mol (1,8 %) ferments after 2 days, while 0,5 mol (4,5 %) shows no development in 6 days.

All series were in fermentation on the 2nd day. The onset of the fermentation was different. In the A series fermentation started only in 0,5 % on the 1st day, in the B series all started, and in the C series 0,5 and 1 % started on the 1st day. The number of yeast cells was estimated after 24 hours with the following result:

Concentration	0	0,5	1,0	1,5	2,0	2,5	3,0
A .....	10	6	2	2	1	1	0
B .....	10	10	10	10	10	8	3
C .....	10	10	7	2	1	1	1

d. *Potassium cyanide* ( $^{29}/_6$ , 45). — In order to illuminate the question whether the differences in reaction of the yeast under various conditions are caused by a more specific resistance to such substances as may occur normally in the metabolism of yeast, or by a more general power of resistance to environmental disturbances which may affect the yeast cell so to say more unexpectedly, an experiment similar to the preceding ones was performed with potassium cyanide.

The reaction of yeast to this substance was tested in advance in a series of 13 concentrations of potassium cyanide, ranging from 0,0001 to 1 mol (0,0005 to 6,5 weight %). The experiment was performed in FREUDENREICH flasks with 10 cc of wort in each. No fermentation occurred after one day in any concentrations. After 2 days 0,0001—0,001 were in full fermentation, 0,002 mol contained living cells, while 0,005 mol and higher concentrations seemed to be killed. Since potassium cyanide may induce both c-mitosis and c-tumours in *Allium* (LEVAN, unpublished), it was of special interest to look for cf-forms in these treatments. In 0,002 mol certain tendencies to simple cf-forms were seen, but hardly in such a frequency that I would care to draw any conclusions.

The experiment with different yeast types was arranged with the following concentrations of potassium cyanide: 0,001, 0,002, 0,003, 0,005, 0,007, 0,01 mol, thus, a scale reaching considerably above the lethality limit of the preceding experiment, in which a yeast from the fuzzy heads stage had been employed.

The differences found between the different yeast types were striking. The A series agreed with the above explorative series: No fermentation on the 1st day, fermentation in 0,001 and 0,002 on the 2nd

day, 0,003 on the 3rd day. On the 4th day 0,003 fermented vividly, while fermentation had finished in 0,001 and 0,002, which were already completely fermented. The microscopic control showed that in the three higher concentrations which did not show any fermentation 0,005 had a beginning development, 0,007 contained living cells, while 0,01 mol seemed killed.

The B series behaved quite differently: On the 1st day fermentation already started in the 4 weakest concentrations, 0,001—0,005 mol, and on the 2nd day all concentrations were well started. On the 4th day the 4 weakest concentrations were finished, while the 2 strongest were in full fermentation. A definitively greater tolerance towards the toxic effect of potassium cyanide was thus demonstrated in the B yeast than in the A yeast.

The C series resembled the A series. In C 0,005 mol also came into full fermentation on the 4th day, while 0,007 and 0,01 never started fermentation and nor did they show any cellular development. Although giant cells were not infrequently met with in these treatments, real cf-cells were very seldom seen.

Summarizing the results of these experiments, it may be stated that in the determination of different threshold concentrations it is of great importance to employ a yeast type which is not only genetically uniform but which is also, as far as possible, in the same physiological condition. The same genotype reacts highly differently if it is in brisk division and full fermentation, if it is at rest after completed fermentation, or, finally, if it has been stored for a long time under conditions favouring autolysis. The few orientating experiments recorded above all give the concordant result that yeast at the stage of the fuzzy heads is most sensitive to noxious effects, while the yeast is most resistant immediately after finished fermentation. The difference in reaction shown between those two types of yeast may represent a change in toxicity threshold from 1 : 2 or 1 : 3. The »geläger» yeast takes an intermediate position in all experiments, the deficient nutrition having evidently exhausted the resistance which it had soon after the end of the fermentation.

## 2. THE INFLUENCE OF THE TEMPERATURE.

As already mentioned in the introduction, it has for long been known that beer yeast has a tendency to grow out to more elongated cells under cold conditions and to form more rounded cells in warmth. This elongated growth in cold may sometimes pass over into an un-

doubted tendency to cf-growth. The detailed conditions under which yeast may give cf-reaction induced by cold are, however, not known.

In order to study the influence of the temperature factor on the cf-reaction I arranged some experiments in February, 1944. They gave the decided result that cold has a general tendency to decrease the threshold value of the cf-reaction induced by various chemicals, while warmth has a tendency to heighten it. These experiments were made in the same manner as already described above. 100 cc of wort with various concentrations of the substances were inoculated with yeast from cultures in vivid fermentation. Three series of each substance were cultured, each of them being kept in a thermostat under the following temperatures respectively:

E: 9°, F: 18—20°, G: 30°.

Since the cf-reaction depends on the developmental stage of the culture — it is easiest to study in the beginning of the growth — the comparison between the three series could not be made after the lapse of the same time. F and G were examined on the 1st day after the inoculation, E on the 2nd to 4th day.

a. *Ethanol* ( $\frac{4}{2}$ , 44). — Concentrations: 1, 2, 4, 6, 8 volume % (0,2—1,3 mol). The lethality is here as in all the other temperature experiments especially high in the highest concentrations of the G series. It is clear that the same concentration of a narcotic substance acts more destructively at 30° than at lower temperatures. The average number of cf-forms in  $\frac{1}{25}$  mm<sup>5</sup> was counted in the three series. It is given together with the percentage of cf-forms calculated on the number of living cells (values within brackets):

Concentration:	1	2	4	6	8	Average
E . . . .	12,4 (74)	10,8 (77)	6,0 (83)	7,6 (86)	0,4 (17)	7,4 (67)
F . . . .	6,0 (30)	8,4 (70)	10,8 (96)	7,6 (79)	1,6 (27)	6,9 (60)
G . . . .	0,3 (0,5)	3,2 (1,6)	3,2 (44)	0,8 ( 6)	0	1,6 (10)

The frequency of cf-forms is evidently lower in the G series. To this comes the fact that both the cell-shape in general and that within the cf-forms were shorter and more rounded in this series. The cf-forms could often not be identified from their cell-form but only from their property of not falling apart when shaken.

b. *Tertiary butanol* ( $\frac{5}{2}$ , 44). — Concentrations: 0,2, 0,6, 1,0, 1,4, 1,8 volume % (0,02—0,2 mol). The following number of cf-forms was recorded:

Concentration:	0,2	0,6	1,0	1,4	1,8	Average
E . . . .	9,6 (55)	8,4 (64)	9,6 (96)	12,0 (97)	12,0 (100)	10,3 (82)
F . . . .	10,8 (35)	8,8 (43)	14,4 (82)	12,6 (97)	20,4 (98)	13,4 (71)
G . . . .	0	6,8 (4)	11,6 (17)	11,2 (52)	6,4 (53)	7,2 (25)

When the percentage of cf-forms on the total number of living cells is calculated, it appears that the curves of the three types of treatment run rather parallelly: E uppermost, G lowest. They all have their main increase in the same concentration region, 0,6—1,4 %.

In this experiment the difference in appearance of the cf-forms

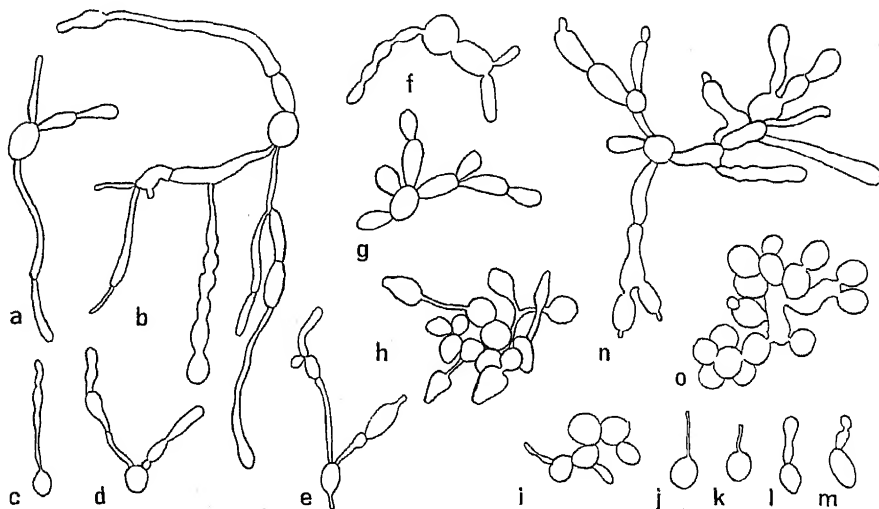


Fig. 6. *a—h*. cf-growth induced by tertiary butanol at different temperatures; *a*: 0,6 % for 3 days at 9, *b*: 1 % for 2 days at 20, *c—e*: 1,4 % for 20 hours at 20°, *f—h*: 1,4 % for 20 hours at 30°; *i—m*: ethyl ether 1 % for 2 days at 30°; *n*: cf-colony from yeast grown on ordinary wort and moved into 1,5 % tertiary butanol, *o*: cf-colony from yeast pre-treated for 15 days with tertiary butanol, thereafter moved over to 1,5 % tertiary butanol. The cell-shape of this cf-form is not elongated. —  $\times 500$ .

originating from the different series was even more striking than in the ethanol series. In E and F the cf-forms had the appearance earlier described with long narrow, tube-like cells (Fig. 6 *a—e*); in G, on the other hand, a more rounded cell-shape predominated (Fig. 6 *f—h*). Often the cell-size of the rounded cells as well is larger than normally. One such rounded cell may send out radiary tubes, all of which soon swell up forming new rounded cells. These colonies may assume fantastic forms with alternating narrow tubes and swollen, spherical or pear-shaped cells (Fig. 6 *h*).

c. *Ether*. ( $\frac{7}{2}$ , 44). — Concentrations: 0,0001—0,05 mol. The lethality was considerable in all three series, only in 0,05 mol at 30° was it almost total. The average lethality of the three series was:

E: 28,9 %, F: 14,7 %, G: 26,0 %.

The increase in cell number was about the same in most cultures; only the weak concentrations of the warmth series showed a much stronger cell proliferation.

In E and F cf-forms were found all through the series, in G they were much rarer and of a less extreme type (Fig. 6 *i—m*). The following numbers were counted in  $\frac{1}{25}$  mm<sup>2</sup>:

Concen- tration:	0,0001	0,0002	0,0005	0,01	0,02	0,05	Mean
E ....	6,8 (42 )	6,4 (48)	5,2 (68 )	4,8 (80)	5,6 (70)	2,4 (50)	5,2 (59 )
F ....	3,2 (18 )	2,8 (14)	3,6 (15 )	6,0 (30)	6,0 (52)	0,8 (18)	2,7 (25 )
G ....	0,4 ( 0,2)	0 ( 0)	1,6 ( 0,1)	0,8 ( 1)	0 ( 0)	0,8 (20)	0,6 ( 3,6)

d. *Urethane* ( $\frac{9}{2}$ , 44). — Concentrations: 0,01—0,5 mol. The lethality was high (97 %) only in the highest concentration of the G series. On the other hand, the cell number counted after 20 hours was especially high in the three lowest concentrations of F and G. In E the cell number had about the same order of magnitude in all concentrations when counted on the 2nd day.

The cf-forms were commonest in E, where all concentrations except the lowest one had them. In F they occurred in 0,1 and 0,2 mol in rather high frequency, in G only in 0,2 mol. The threshold may be said to vary with the temperature in the following manner:

9° .....	0,02 mol
18—20° .....	0,1 »
30° .....	0,2 »

The round cell-form of the cf-forms of urethane may have made the recognizing of all cf-forms difficult. In the F series, too, the shape of the cells was mostly quite round. The following numbers of cf-forms were found (percentages in brackets):

Concen- tration	0,01	0,02	0,05	0,1	0,2	0,5
E ....	0	2,8 (20)	3,6 (15)	7,2 (75)	0,4 (14)	—
F ....	0	0	0	24,0 (58)	2,8 (64)	0
G ....	0	0	0	0	3,2 (73)	0

All these experiments on the cf-reaction under influence of different temperatures, however preliminary, show the same general tendency. The induction of the cf-reaction is facilitated by low temperature and is impeded by high temperature. Since in some cases low temperature alone has evident tendencies to favour cf-growth, it may be concluded that cold adds to the effect exerted by the various chemicals. Therefore a lower concentration is sufficient under cold conditions to get the same effect as, at a higher temperature, would have been induced by a higher concentration.

### 3. INDUCED RESISTANCE TO THE CF-REACTION.

One feature common to all treatments of yeast with narcotic substances is that the threshold of toxicity as well as of the cf-reaction does not remain constant during the course of the experiment but has a tendency gradually to rise. Such concentrations of a substance as during the 1st day give only weak development in cell number and high frequency of cf-forms often give during the 2nd day full development in cell number and now preponderantly normal cells and very few cf-forms. Evidently something happens which brings about either a decreased concentration of the substance in the culture medium or a greater tolerance (= increased threshold) of the yeast cells towards the treatment.

Since this peculiarity of an upward shift of the thresholds during the experiments is characteristic of all the different types of treatment, it is *a priori* hardly probable that the yeast cells have the ability through their own metabolism to remove or otherwise make harmless such a proportion of the various substances that the concentration which they have in the surrounding medium will fall below the threshold value. The other alternative, which involves an acquired resistance of the yeast cells, seems more plausible, and is in agreement with the conditions of many other types of narcotic and toxic reactions. Several experiments have been arranged to test whether the culturing of yeast in a medium containing a certain substance makes the cells more resistant to this substance, and perhaps also to other substances. Broadly speaking, all experiments indicate that such is the case. However, many disturbing factors are at work in these types of experiments, so I cannot as yet consider the problem as solved. I hope to continue the work along this line, and for the present I will only mention in all brevity one preliminary experiment made in February, 1944. This may indicate one line of approach to this important problem.

A number of yeast cultures were given various pre-treatments. They were grown for a fortnight on wort containing such concentrations of various substances as initially brought about a strong checking of the propagation of the yeast and also gave a high frequency of cf-forms but as later on permitted a good development of the yeast and full fermentation. These solutions were the following:

1. Ethanol ..... 1,2 mol
2. Tertiary butanol.. 0,2 »
3. Lauryl alcohol .. 0,01 » (saturated, with excess of substance)
4. Acetone ..... 0,41 »
5. Ether ..... 0,19 »
6. Chloroform ..... 0,0063 »
7. Paraldehyde .... 0,15 »
8. Pure wort as control

Yeast from these cultures was now inoculated on wort with three of the above substances, viz. ethanol, acetone and ether, in the above concentrations. The following estimate of the fermentation and the frequency of cf-forms was made on the 3rd day:

Pre-treatment	Ethanol		Acetone		Fermentation	Ether	
	Fermentation	Cf-forms	Fermentation	Cf-forms		Cf-forms	
Ethanol .....	+	1	±	7	—	no development	
Butanol .....	+	0	—	0	—	»	»
Lauryl alcohol ..	±	0	+	0	—	»	»
Acetone .....	+	1	++	0	±	2	
Ether .....	—	0	±	1	—	no development	
Chloroform .....	+	2	—	0	—	»	»
Paraldehyde ....	+	1	±	2	—	»	»
Pure wort .....	+	5	+	10	—	»	»

Some interesting facts are seen from this. The acetone-yeast gives the strongest fermentation in the acetone-series, and is the only one which shows any development at all in the ether series. Although the control yeast gave a high frequency of cf-forms in acetone, the acetone-yeast had no cf-forms. The development of the cf-forms was throughout more extreme within the control yeast than in any of the pre-treated yeasts. As an instance of this Fig. 6 n shows a typical cf-form from the control yeast in 1,5 % tertiary butanol, while Fig. 6 o shows a cf-form of the butanol-yeast under the same treatment. The former cf-

colony has the shape typical of such a treatment, while the latter, although it has formed a cf-colony, consists of normal cells.

These results point to an evident unspecificity of the induced resistance. All kinds of pre-treatments have resulted in some degree of resistance to ethanol and acetone, the untreated yeast giving the most extreme cf-growth in both series. It must be stated that the experiments on pre-treatments of yeast with different substances point in the same direction as tests of the yeast in different physiological conditions. As in these experiments, here too, a certain unspecific resistance may be obtained by the yeast.

### VIII. CONCLUSIONS.

The present morphological and experimental work on the cf-reaction of yeast leads to the following points and conclusions:

(1) The very type of the reaction suggests a disturbance or an irregularity of the growth, a switching-off of the growth control, which under normal conditions is responsible for a regulated cellular growth and propagation.

(2) This switching-off of the growth control may be more or less complete: in some cases the entire growth results in long irregular threads or swollen sacs, in other cases a certain growth pattern is maintained, recalling the morphology of other *Saccharomyces* species. By this fractionated withdrawal of different elements of the growth control a certain analysis of the normal growth may be attained.

(3) The cf-reaction is reversible: if the concentration of the active substance in the culture medium sinks below the threshold value of the reaction, or if the yeast cells acquire resistance to the treatment, the same cells as were earlier under command of the reaction, start normally to bud off daughter cells, which in the sequence behave normally.

(4) The nuclear divisions of the cf-forms are to a certain degree independent of the cellular disturbances. Normal mitoses may be found during a prolonged cf-growth. However, nuclear disturbances also gradually appear in strong concentrations of various substances. The commonest types of these disturbances are the following: a considerable increase in chromosome number of the nuclei, irregularities in the mitotic mechanism, multipolar spindles, vagabonding chromosomes, origin of micronuclei, etc. Whether regular c-mitoses occur is still a matter of doubt. The presence of tetraploid nuclei in otherwise normal cells, which was observed after rather short treatments, makes it per-

ceptible that the threshold of c-mitosis in these organisms lies close to the lethality limit. It is possible that in strong treatment single c-mitoses are immediately induced, thereupon followed by an acquired resistance, which moves the c-mitotic threshold above the lethality limit. The nuclear disturbances appearing later on have the same general type as weak c-mitotic disturbances in which the spindle is made somewhat ineffective but is not totally narcotized. The high chromosome number often seen in the nuclei of old cf-cells, as well as the long resting stage which these nuclei are seen to go through, indicate endomitotic processes. In the same manner as is known for higher plants and animals the enormous increase in cellular volume may bring about an adjustment of the chromosome number of these cells.

It may be stated that parallel with the disturbed control of the cellular growth, though not necessarily dependent thereon, disturbances also occur in the mitotic control, disturbances of the same type, but less complete than the c-mitosis of higher plants.

Certain types of induced mitotic disturbances made it possible to observe the size of single chromosomes. This condition gave a key to the normal chromosome number of the yeast studied, which was estimated to at least ten, possibly higher.

(5) The mitotic disturbances are also reversible. If an increase in chromosome number has taken place, the higher number is of course maintained, but after the end of the treatment the normal course of mitosis is restored. It is true that in SANDWALL's and my experiments no permanent polyploid yeast strains have been isolated, which is probably due to the fact that relatively little work has been done by us in that direction. Yeast types with in all probability polyploid chromosome number have originated on several occasions in the work of other authors.

(6) The cf-reaction is not confined only to a few certain chemicals, camphor and some derivatives of camphor. In the present experiments it has been induced by some fifty substances, aliphatic, alicyclic and aromatic. Most of the substances are of the type known for their narcotic properties.

This fact alone indicates that the reaction is not tied to any certain chemical constitution. It rather suggests that certain physical properties common to the efficient substances may be of significance.

(7) This conclusion is strengthened by the persistently observed correlation between physical properties (water solubility) and threshold value of the reaction. The reaction is generally induced by concen-

trations which lie in the vicinity of the saturation point of the substance, only seldom does it go below 0.1 of this concentration. According to the view of FERGUSON (1939), this corresponds to a thermodynamic activity of 1—0.1, which is characteristic of unspecific, physical action.

Although this condition may be the rule, it does of course not exclude the fact that some substances give effect at decidedly greater distances from the point of saturation. The lower aliphatic alcohols, acetone, chloral hydrate, chloroform and many others may have thermodynamic activities indicative of a more specific, chemical action.

It is possible that the common features of the reaction are caused by a physical action common to all the acting substances. The manifold deviations in type of the reaction are perhaps induced by specific chemical groups in the substances.

(8) A certain unspecific resistance to the reaction may be induced. Cells which have gradually been accustomed to an increasing content of ethanol (yeast from the sediment immediately after completed fermentation) are found to be more resistant not only to ethanol and butanol, but also to potassium cyanide, than cells which have for a long period grown only on wort without any considerable content of ethanol.

(9) Cold, alone or in combination with chemical treatments, imparts to the cells increased tendencies to give the cf-reaction.

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Through these studies the cf-reaction, which has hitherto occupied rather an isolated position, has been brought in line with the also normally occurring phenomena of involution growth of yeast and of the morphological deviations in cell type induced by cold and other environmental conditions. Furthermore, these phenomena are now comparable to certain other reactions of living organisms, caused by various external factors. For instance, the cf-growth of the cells is of similar kind to the c-tumour reaction of higher plants, the nuclear disturbances of the cf-reaction are comparable, if not identical, with the c-mitotic reaction.

The cf-reaction may consequently be regarded as a partial reaction of the important complex known as the narcotic reactions. Common to all of these is the reversible switching-off of higher, controlling functions, the unspecificity of the induction, the correlation with physical properties of the inducing substances, the possibility of acquiring resistance. In addition to the general narcotic reactions of animals and

the more specific reactions just mentioned of c-tumour, c-mitosis, cf-growth, there also belong here many forms of general toxic action. Common features are easily found between the narcotic reactions and several bactericidal and insecticidal reactions, as has often been pointed out in the literature. If these narcotic-toxic reactions are allowed to extend their action so far that vital functions are held up, which cannot be switched off without serious damage, death by narcosis will be the result.

The individual character of each of the more specific reactions as, for instance, the cf-growth is given through the interrelation of their activity thresholds with toxicity and lethality. The greater the distance is between the threshold of the specific action, on one hand, and toxicity and lethality, on the other, the easier is the reaction obtained, the more extreme is its expression, the more efficient is the inducing substance said to be.

Some persistent features pointed out in connection with certain groups of related substances treated above, either homologous series, as the aliphatic alcohols, or derivatives of a certain substance, as the camphor group, may receive some illumination from these conditions. It has been mentioned that in such groups of substances an optimum may exist for the induction of the reaction. Thus, among the alcohols, butanol and pentanol represented the optimum, in the camphor group, camphor. Towards both sides substances occurred which gave the reaction less completely and with greater difficulty. The nature of these optima may be understood when viewed in relation to the different reaction limits just dwelt upon. At the optimum the maximal distance must be present between the activity threshold and the toxicity threshold.

On moving within the series towards the lower (=more water soluble) members (in our instance towards methanol or camphoric acid), the activity threshold will move upwards, higher concentrations will be needed to attain the effect. The unspecific poison effect also shifts upwards, but here there appears a specific toxicity, known, for instance, for the lower alcohols from other biological reactions. The curve of the toxicity threshold will consequently bend downwards and converge towards the activity threshold. This may be the cause of the diminished activity in the lower members of a series.

On moving in the opposite direction from the optimum, that is towards the higher (=less water soluble) members of the series, the activity threshold, as well as the toxicity threshold, sinks, though less

rapidly than does the solubility. Consequently, a point is reached at which the activity line crosses, and passes above, the solubility line, and the same will happen with the toxicity curve. To the left of this point the solubility will be too low to give the reaction, or, as the case may be, to give poison effect, the well-known cut-off of activity (cf. FERGUSON, l. c.), of toxicity or of lethality is reached. In our instance the cf-reaction is cut off between octanol and cetyl alcohol, and between borneol and bornyl acetate. Already at octanol it was noticed that the toxicity threshold, the cf-threshold and the solubility threshold were crowded together, resulting in a very narrow amplitude of the cf-reaction. In cetyl alcohol neither the cf-reaction nor any high degree of toxicity was obtainable. In the camphor group, on the other hand, even the least soluble substances had lethal effect in some concentration. Here the toxicity line had not yet crossed the solubility line, this point of crossing in the case of camphor derivatives evidently lying far to the left from the point of the cut-off of the cf-reaction.

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# THE CYTOLOGICAL EFFECT OF CHLOROFORM AND COLCHICINE ON ALLIUM

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## I. INTRODUCTION.

BY introducing the concept of thermodynamic activity into toxicology FERGUSON (1939) arrived at a division of the various poisons into two classes, those with preponderantly physical and those with preponderantly chemical action. Since thermodynamic activity of a substance in solution may be roughly expressed as the relation between the lowest active concentration and the solubility, it is important to know the solubility of substances which are employed as agents.

The information given in the literature concerning the water solubility of colchicine is rather varying. According to the fifth Swiss Pharmacopoeia, one part of colchicine may be dissolved in 70 parts of water. The Handbook of Chemistry and Physics (1944) gives the water solubility as 4.5 %, MÜLLER (1903; see LEVAN and ÖSTERGREN, 1943) gives it as 9.6 %, ZEISEL (1886), finally, states that colchicine may be dissolved in water in all proportions, these being only a few instances of references in the literature to the solubility of colchicine. These apparent contradictions receive their explanation in the property of colchicine to form crystals which contain various solvents. As early a worker as ZEISEL (l. c.) found that colchicine obtained from a chloroform solution will form crystals with a certain content of chloroform. Such a type of colchicine will show a decreased water solubility. By heating with water the compound is again split into its components. On evaporating the water solution the chloroform disappears and a chloroform-free colchicine is obtained. If a strong colchicine solution is left for some time, the alkaloid precipitates with crystals containing  $\frac{1}{2}$  mol of crystal water. This form is the one referred to in the Swiss Pharmacopoeia, and the solubility 1 : 70 is valid for this form. Over sulfuric acid in a vacuum the crystals gradually lose their water and change into the form free from solvent, which form is of unlimited water solubility. The solubility of this amorphous colchicine decreases, according to ZEISEL, with increasing temperature: thus at 82° it is only

12 %. The following forms of colchicine may be recorded together with their water solubilities:

Pure (amorphous) colchicine	unlimited
Colchicine + $\frac{1}{2}$ mol $H_2O$	1 : 70
Colchicine + $\frac{1}{2}$ mol $CHCl_3$	about 8 %
Colchicine + 1 mol $CHCl_3$	about 4 %

The content of solvent in colchicine is of no great consequence for the practical polyploidizing experiments, although it is probable that the chloroform-containing form is more poisonous than the pure colchicine, and therefore may be somewhat less suitable for some purposes. On the other hand, these conditions must be of great importance when quantitative investigations on the effect of colchicine are concerned. Thus, colchicine with 1 mol chloroform contains 25 % by weight of chloroform. Only in exceptional cases have the workers in this field given information as to what kind of colchicine has been employed, and only rarely has a direct comparison been made between the different forms of colchicine. BRUES and COHEN (1936), in their study of the active group of the colchicine molecule, test chloroform-containing and chloroform-free colchicine on regenerating rat liver. They obtained from the two treatments »entirely similar results as far as the cytological picture was concerned». BOAS and GISTL (1939) point out that in higher concentrations of crystalline colchicine the chloroform content cannot be disregarded. MANGENOT (1942) compared crystalline and amorphous colchicine in concentrations of 0,05 and 0,025 % without observing any differences in the effect on *Allium* roots. MAIOLD (1943) found no difference in the influence on plasmolysis between the two forms of colchicine. On the other hand, a distinct difference was shown in the effect on the growth of *Lupinus* roots of very low concentrations. While 0,001 % of amorphous colchicine acted stimulating on the root growth, the same concentration of crystalline colchicine gave only growth retardation. At higher concentrations as, for instance, 0,005 % both forms gave an equal retardation. MAIOLD assumes that in this case only very weak concentrations allow the effect of chloroform to come to light, in higher concentrations the effect of colchicine will pre-dominate. MAIOLD cites a work of JOSING (1901) which showed that also in very low concentrations (as low as 0,0001 %) chloroform may have an effect on the plasmatic current.

In the course of our studies of c-mitotic effects on various liliaceous plants we have directed some attention to differences in action of

amorphous colchicine and crystalline colchicine. This point has been tested both in plants sensitive to colchicine and such as are known for their resistance to this substance. In the present paper only the first case will be considered, the effect of chloroform alone, chloroform-containing colchicine and chloroform-free colchicine being studied. As experimental object root-tips of *Allium Cepa* have been employed.

## II. THE EFFECT OF CHLOROFORM.

The chloroform employed was immediately before use deacidized with sodium carbonate, thereafter washed with distilled water until neutral reaction was given, dried over  $\text{CaCl}_2$  and fractionated. A series of eight concentrations from saturated solution (1 % by weight) down to 0,005 % was studied. As regards the cytological effect, the two strongest solutions 1 and 0,5 % were of considerable interest and must therefore be described in some detail. In these concentrations the roots rapidly became soft. Cytologically the picture suggests a fixation effect, the various mitotic stages were kept as they were at the moment the root-tips were killed. No c-mitotic effects occurred, nor any reversions of the contraction stages into despiralized stages.

The appearance of the resting nuclei was striking (Fig. 1 *a, b*). All through the meristem the resting nuclei were provided with irregular outgrowths into the plasm, resembling the precipitation membranes of »chemical gardens». These protuberances, as also the entire nuclei, were often quite devoid of any chromosomal structures. Just the centromeric heterochromatin was, however, often left (Fig. 1 *a, b*). In some nuclei chromosome threads occurred, these often being extended through the nuclear protuberances into the plasm (Fig. 1 *c*).

The contraction stages of the chromosomes, metaphase—anaphase, are characterized by a gradually proceeding dissolution of the chromatic material. In some pictures (Fig. 1 *d*) it was clearly seen that the matrix material left the chromosomes and wandered out into the plasm. The chromonema, or genic thread, apparently showed a greater resistance to the dissolution. Often these parts of the chromosomes were extremely delicately stained, giving an unusually clear picture of the internal structure of the chromosomes. Although the structures thus revealed must be considered as lethal, they are according to our opinion of a certain significance for the understanding of the vital structures. Fig. 1 *e* shows one cell at early metaphase, where most of the matrix is melted away, *f—j* show other instances of metaphase chromosomes,

*k—n* are anaphase chromosomes. A feature common to all these pictures is the subdivision of the chromosomes into chromatids, half-



Fig. 1. *a—c*: resting nuclei treated with 0,5 % chloroform for 4 hours; *d*: metaphase, same treatment; *e—j*: metaphase, *k—n*: anaphase chromosomes,  $\frac{1}{2}$  hour in 1 % chloroform; *o—q*: metaphase; *r—s*: anaphase, 4 hours in 0,5 % chloroform; *t*: bow metaphase, 4 hours in 0,25 %; *u*: c-metaphase with strong chromosome contraction, 24 hours in 0,1 %; *v*: c-anaphase, 4 hours in 0,025 % chloroform. —  $\times 1500$ .

chromatids and often also  $\frac{1}{4}$ -chromatids. Especially far-going is this morphological sub-division towards the chromosome ends, which often show signs of many threads (Fig. 1 *f*).

Certain metaphase chromosomes were considerably swelled (Fig. 1 *i, j*). They were very faintly stained, most of the chromatic substance already being washed out. Through the staining many fine threads were visible in longitudinal direction, clearly indicating a cable structure of the chromosomes. By the treatment evidently the many single threads, which are usually glued thickly together, had been loosened from each other. A similar division in two and more threads was present in the anaphase and telophase chromosomes (Fig. 1 *k—n*). The daughter half-chromatids were in these cases either turned in a relational spiral or were comparatively free from each other. Often definite chromomeres were visible, chromomeres of similar appearance occurring on the same level within the two half-chromatids.

In one slide (treated with 0.5 % for 4 hours) the dissolution of the chromosomes had often gone still one step further. Similar pictures were frequent in this slide as have earlier been found by ÖSTERGREN to occur after other narcotic treatments (unpubl.). Of the chromosomes there remained only the centromeric apparatus, the chromosome arms having completely disappeared (Fig. 1 *o—s*). Evidently the centromeric region is the most resistant part of the chromosomes. It was already known that this region often gives staining reactions different from the rest of the chromosomes (LEVAN, 1946, and literature given there). In the present case not only do the centromeric parts appear more darkly stained than the chromosome arms, but the latter are often totally absent. Fig. 1 *o* shows this condition in metaphasic polar view, *p—s* shows it from the side, at metaphase (*p—q*) and anaphase (*r, s*). It is clear that this condition gives a rare occasion to submit the centromeric apparatus to a close morphological scrutiny. Owing to the smallness of all details, however, caution must be used in the interpretation of the structures seen. It seems to be certain, however, that the centromeric apparatus is a complex structure, as has indeed been supposed by most workers in this field. The pictures show partly the heterochromatic lateral segments (the prochromosomes), partly one small body (the spindle spherule) at which the spindle fibres seem to attach. The spindle spherules at metaphase are often extended towards the poles and protrude somewhat above the surface of the chromosomes. At anaphase the spindle spherule is often the largest part of the whole centromeric apparatus present. Besides this round body, only small

irregularly shaped remnants are visible, directed from the poles (Fig. 1 *r, s*). The spindle spherule, on the other hand, showed about the same size in all chromosomes of one cell. No traces of the chromosome arms besides the above irregular remnants were seen.

These strong concentrations of chloroform have an immediately killing effect, the cells being fixed. A gradual dissolution takes place after the killing, which first affects the matrix, later on the whole chromosome arms, the centromeric part showing greater resistance than the rest of the chromosomes.

The next concentration, 0,25 %, is not directly lethal. The roots keep turgescient for about 4 hours, but are quite soft after 24 hours. In 0,10 % the roots remain turgescient several days. The cytological picture is here quite different. A certain general stickiness occurs, but the chromosomes are not dissolved to the same extent as in the two strongest concentrations. Furthermore, the cells keep alive long enough to show c-mitosis, which in these concentrations is the only mitosis type already occurring after four hours. A strong toxic effect is noted; after 24 hours almost only resting nuclei occur, no new mitoses being started.

C-mitosis goes down to the concentrations 0,05 and in solitary cells to 0,025 %, dubious tendencies to c-mitosis may be found even in 0,010 %. In these concentrations, however, normal mitoses predominate. The c-mitosis is of quite the ordinary type. Often the type of »bow-metaphase» (LEVAN, 1946) occurs (Fig. 1 *t*), which is characterized by the chromosomes being scattered widely over the cell, each chromosome showing little contraction, its chromatids being tightly glued together, the entire chromosomes often being somewhat arched. Especially in the few mitoses occurring after 24 hours' treatment the contraction of the c-pairs might be maximal (Fig. 1 *u*), each chromosome approaching the spherical shape. The c-anaphases (Fig. 1 *v*) were typical.

Clear c-tumours were found only in two concentrations, 0,05 and 0,025 %, the higher ones evidently being too poisonous to allow c-tumour growth. Weak tendencies to c-tumours were also seen in 0,010 %, but only in some roots.

Our results on the chloroform effect in *Allium* may be summarized as follows:

Concentrations		Roots turgescient	Dissolution of chromosomes	C-reactions	
%	mol/l			mitosis	tumours
1	0,08	—	+	fixing	—
0,5	0,04	—	+	fixing	—

Concentrations %      mol/l		Roots turgescnt	Dissolution of chromosomes	C-reactions mitosis      tumours	
0,25	0,02	—	sticky	+	—
0,10	0,008	+	sticky	(+)	—
0,05	0,004	+	—	±	+
0,025	0,002	+	—	(+)	+
0,010	0,0008	+	—	—	(+)
0,005	0,0004	+	—	—	—

### III. THE EFFECT OF COLCHICINE WITH AND WITHOUT CHLOROFORM.

On indicating the concentration of colchicine solutions used in most earlier investigations a certain weight percentage has been given; no regard has been taken to whether the colchicine was pure or contained chloroform. If molar concentrations have been used, 399,32 has been employed as molar weight of colchicine, which is correct only for the pure amorphous colchicine. It is therefore of the greatest significance to test the critical threshold region within both types of colchicine in order to investigate whether differences occur which may necessitate an adjustment of threshold values earlier given. As mentioned in the introduction, some earlier studies suggest that no great differences in effect are present in the low concentrations under consideration here. Thus, LEVAN (1938), probably using amorphous colchicine, found the threshold value of c-mitosis in *Allium* to lie between 0,005 and 0,01 %. LEVAN and ÖSTERGREN (1943), with crystalline colchicine, determined the same value to be 0,004—0,006 %.

In the present experiment the following substances were used: (1) Colchicin. puriss. amorphous Sandoz, Basle, and (2) Colchicin. puriss. cryst. +  $\frac{1}{2}$  mol  $\text{CHCl}_3$  Merck, Darmstadt. The colchicine substances were tested for chloroform according to the isonitril-reaction (Fifth Swiss Pharmacopoeia): 2 cg colchicine, 2 cc 2-normal NaOH and 1 drop aniline are heated together. If chloroform is present the typical smell of phenyl isonitril is noticed. Colchicine (1) turned out to be completely free from chloroform, while (2) gave a strongly positive reaction. Of both substances a series from 0,002 to 0,02 % by weight were tested. Fixations of the root-tips were made 4 and 24 hours after starting the treatment. The results as far as c-mitosis and c-tumours are concerned are given in Table 1.

From this table it is seen that both series behave perfectly alike.

TABLE 1. *Comparison between different forms of colchicine.*

Weight %	Amorphous colchicine ( $C_{22}H_{25}NO_6$ )				Crystalline colchicine ( $C_{22}H_{25}NO_6 + \frac{1}{2} CHCl_3$ )			
	Molarity of colchicine	c-mitosis		c-tu- mours	Molarity of colchicine	c-mitosis		c-tu- mours
		4 hours	24 hours			4 hours	24 hours	
0,02	0,00050	+	+	++	0,00043	+	+	++
0,01	0,00025	+	+	+	0,00022	+	+	+
0,007	0,000175	±	±	+	0,00015	+	±	+
0,004	0,000100	±	±	±	0,000087	±	±	±
0,002	0,000050	—	—	—	0,000043	—	—	—

The agreement is further accentuated by Fig. 2, which shows the c-tumour reaction of the two series. Not only are they alike in the general type of the reaction but also in the smallest details. The tumours of 0,02 % are, for instance, of similar size and shape in both

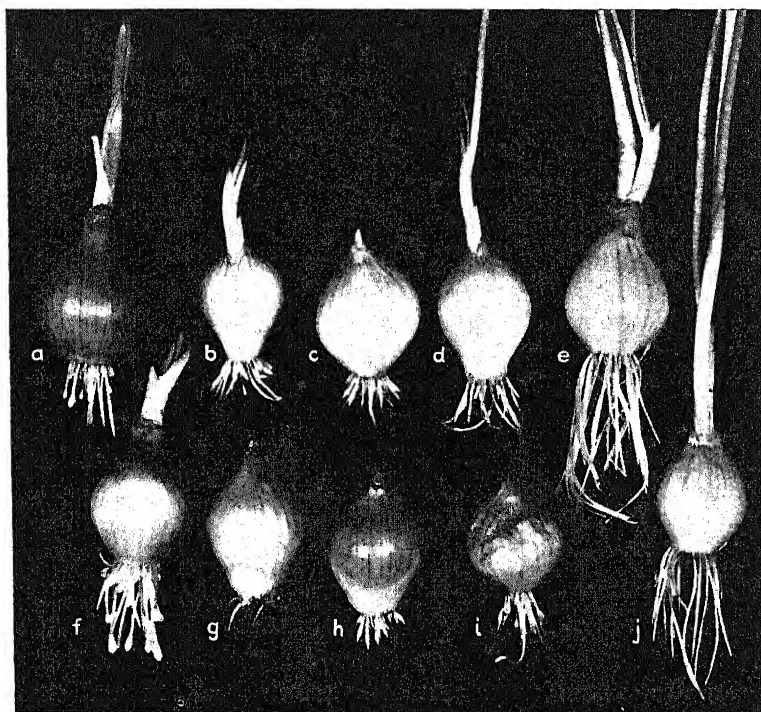


Fig. 2. The c-tumour reaction in chloroform-containing colchicine (*a—e*) and in pure colchicine (*f—j*); *a, f*: 0,02 %, *b, g*: 0,01 %, *c, h*: 0,007 %, *d, i*: 0,004 %, *e, j*: 0,002 %.

series, and are somewhat distinguished from the tumours of the lower concentrations. The limit concentration 0,004 % in both series starts typical tumour growth, but after some time tumours are overgrown by normally elongating root-tips.

The experiment has shown that in this concentration range it is quite without consequence which form of colchicine is used. Earlier determined threshold values may thus be relied upon independently of what kind of colchicine has been used.

#### IV. CONCLUSIONS.

The above experiments have shown that chloroform gives typical c-mitotic and c-tumour reaction in concentrations down to 0,025 % by weight (0,002 mol/l). In crystalline colchicine, where chloroform is present in fractions of  $\frac{1}{4}$  or  $\frac{1}{8}$ , concentrations of 0,1 or 0,2 % would be needed to reach the threshold value of chloroform. In the concentration range of colchicine giving c-mitosis in *Allium*, 0,002—0,004 %, there is evidently no risk that the chloroform will have any c-mitotic effect. The small deficit of colchicine in the crystalline form is not sufficient to exceed the limits of normal experimental error.

The thermodynamic activity of chloroform may be determined according to our results; it is calculated as the relation of threshold value to water solubility. As a comparison the same values for ether are given, the threshold for c-mitotic action being taken from ÖSTERGREN (1944):

Substance	Solubility in $10^{-6}$ mol/l	Threshold in $10^{-6}$ mol/l	Thermodynamic activity
Chloroform . . . . .	83'000	800—2'000	0,01—0,02
Ether . . . . .	1'000'000	62'500—125'000	0,06—0,13

A thermodynamic activity of 0,05 is considered to denote the extreme lower limit for a pure unspecific, physical activity of a narcotic (FERGUSON, 1939; GAVAUDAN, DODÉ and POUSSEL, 1945). Since chloroform evidently goes below this limit in our experiments, a certain degree of chemical activity can hardly be denied in this substance. The activity of ether, on the other hand, is well within the limits of physical activity. In this connection it should be pointed out that chloroform undoubtedly had a definite toxic effect on the *Allium* roots as far down as our series went, i. e. to 0,0004 mol. Thus, the mitotic activity was almost completely inhibited after 24 hours in this concentration. It was also noticed that

a weak poison effect was present in some of the tested concentrations of crystalline colchicine which was not present in the amorphous substance. It should be mentioned that chloroform, in contradistinction to ether, is known for a considerable toxicity in its narcotic action on animals. This condition has actually limited its usefulness as an anaesthetic in surgical practice.

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### SUMMARY.

The cytological effect of chloroform as well as of amorphous and crystalline colchicine is studied. Strong concentrations (saturated— $\frac{1}{2}$  saturated solutions) of chloroform bring about a characteristic cytological effect. The chromosomes are dissolved, in extreme cases only the centromeric apparatus is left. In lower concentrations the c-mitotic reaction is found. The threshold for this reaction is 0,0008—0,002 mol/l. The threshold region of c-mitosis in *Allium* in the two types of colchicine was found to coincide, the chloroform present in the crystalline colchicine being too diluted to have any c-mitotic effect. The water solubilities of the substances employed here are discussed and the results of our experiments are put in relation to the thermodynamic activity of the threshold concentrations.

Svalöf, March, 1947.

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# SOME OBSERVATIONS ON THE SEED DEVELOPMENT IN ECUADORIAN CACAO

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PROFESSOR ARNE MÜNTZING has made observations and experiments on pollination and fruit setting in cacao trees at the Swedish Hacienda Clementina in the neighbourhood of Babahoyo in Ecuador (MÜNTZING, 1947). A number of ovaries and young fruits (cherelles) from hand-pollinated flowers were fixed for embryological studies and thus I have been able to study some stages of the seed development in this material. The development of the embryo-sac in *Theobroma cacao* has been studied by KUYJPER (1914); on fertilization and later stages of the seed development there is a good investigation by CHEESMAN (1927). The embryo-sac develops after the normal type, and is rather small. In ovaries fixed five or six hours after pollination pollen tubes could be observed in the micropylar region of the embryo-sac, and two hours later CHEESMAN could sometimes observe male nuclei lying against the egg or the polar nuclei. »The first division in the embryo-sac takes place sometimes on the third but more often on the fourth day after fertilization, and in preparations made then two endosperm nuclei are found» (CHEESMAN, l. c.). Fig. 12 of CHEESMAN shows the second division of the endosperm nucleus 120 hours after pollination. The first cell walls in the endosperm appeared when the fruit was not less than 50 days old. Very slow is the development of the embryo, the youngest embryo observed was in a fruit 50 days old. »The first division of the fertilized egg was not observed, but apparently it occurs from forty to fifty days after fertilization.» CHEESMAN found in nearly full-grown fruits that the embryo may be less than two mm. long, but later the embryo must grow very rapidly, »its development being nearly complete when the fruit stops growing». Parthenocarpy does not occur.

The literature on pollination, fertility and fruit development in cacao is rich (compare MÜNTZING, l. c.). It is a well known fact that the cacao tree produces a very large number of flowers (50.000), of these a varying number set (in one case almost 6.000), while perhaps 100 mature fruits are a good crop. Thus most ovaries are dropped the first week after the flowering, and during some following weeks (3—7)

a variable number of developing pods wilt, the so-called cherelle wilting. The fruit is mature after about 6 months. POUND (1931 a) has observed a constricted region on the pedicel, where the abscission of the flower is brought about by an active osmotic mechanism. POUND thinks that the rapid growth of fertilized ovaries drains the soluble carbohydrates of the pedicel and therefore the osmotic mechanism does not function and the flower is not dropped. There is no self-pollination within the cacao flower. Pollination has been claimed to be effected through the agency of thrips, aphids and red ants. However, the Ceratopogonid midge *Forcipomyia* has been shown to be responsible for most of the effective pollination of cacao flowers in Trinidad (see POSNETTE, 1944). At Clementina aphids, directly or indirectly through ants, contribute to pollination; the spontaneous pollination was, however, very often faulty, many styles having no or only a few pollen grains (MÜNTZING, l. c.). POUND (1931 b) has shown that ovaries from different trees contain about the same number of ovules, while mature fruits have a varying number of beans, 25 % or as many as 50 % of the ovules not having developed into beans. Later (POUND, 1934) it was shown that ovaries six days after pollination may have a varying number of ovules that had not increased in size, these ovules probably being unfertilized. The variation of the bean number in mature fruits is thus largely a result of incomplete pollination, and MÜNTZING finds that this agrees with the conclusions drawn from his own observations. POUND (1931 a) has further shown that the cherelle wilting cannot be due to incomplete fertilization, because an investigation of cherelles having a length of two inches showed that the number of good ovules was about the same in wilted fruits as in good fruits (the cherelle wilting has been attributed to a competition for water and nutrients).

In Trinitario and Criollo cacao there are self-compatible (self-fertile) and self-incompatible (self-sterile) trees. The latter have no or very reduced fruit setting when self-pollinated or pollinated with pollen from other self-incompatible trees; they have good setting when pollinated with pollen from self-compatible trees (see POSNETTE, 1944). COPE (1938) has shown that the incompatibility is not due to an arrested or reduced growth of the pollen tubes. POSNETTE showed that incompatible pollen on the stigma caused reduced setting when mixed with compatible pollen unless the latter had some hours better start or a shorter tube-path to travel. POSNETTE (1945) showed through hand-pollinations that self-incompatible types are very common in Amazonas cacao, although they differ from self-incompatible Trinitario types in

being cross-compatible. MÜNTZING (l. c.) used four trees for his pollination experiments, the trees 2, 3 and 4 were self-incompatible while tree 5 was self-compatible. Cross combinations resulted in a commencing fruit development in the cross self-incompatible  $\times$  self-compatible ( $3 \times 5$  and  $5 \times 3$ ), and in one of the crosses self-incompatible  $\times$  self-incompatible ( $2 \times 3$  and  $3 \times 2$ ), whereas  $3 \times 4$  was quite negative.

An embryological investigation on compatible and incompatible cacao crosses has already been made by COPE (1939). Flowers of a self-incompatible tree were selfed or pollinated with pollen from a self-compatible tree; fixations were made, the latest 96 hours after pollination. Fertilization was the same in ovaries pollinated incompatibly as in those pollinated compatibly; after 30 hours no difference could be observed. Certain post-fertilization differences became, however, more pronounced with lapse of time. Division of polar nucleus was initiated considerably later in incompatibly fertilized embryo-sacs, and at abscission less than 25 % of the embryo-sacs showed divided polar nuclei. While in the compatible cross after 58 hours 22 % of the embryo-sacs had divided polar nuclei, after 72 hours 82 %, the corresponding figures from the incompatible cross were 0 % and 5 %. COPE considers that the late inception and low level of nuclear activity in incompatibly pollinated ovaries are responsible for abscission. POSNETTE (1945) showed that in ovaries of a self-incompatible Amazonas type pollen tubes had entered the embryo-sacs 30 hours after pollination, thus confirming the observations of COPE.

The results from the investigation of the Clementina material were the following. It must be observed that in most cases the content of all ovules in the ovaries (their number is more than forty) could not be studied, because many sections were often lost during the staining of the slides and the starch grains in the embryo-sac often obscured the polar nuclei, making observations on their behaviour impossible.

*Selfing of 5* (compatible pollination). — 10 hours: in one ovary 17 fertilized, 2 unfertilized embryo-sacs were counted, in one 5 fertilized, 12 unfertilized, in one 2 fertilized, 12 unfertilized. In »unfertilized embryo-sacs» both the synergids were intact and no trace of the pollen tube could be detected in the sac. — 24 hours: in two ovaries most embryo-sacs were fertilized, in one all the embryo-sacs were unfertilized. In the latter case the ovules and embryo-sacs had a rather youngish appearance. — 48 hours: two ovaries had very few, one had rather many, unfertilized embryo-sacs. In unfertilized embryo-sacs the polar nuclei are still in the vicinity of the egg-apparatus, in fertilized embryo-sacs they have moved and are often near the chalaza, and one of them often has two nucleoli suggesting fusion

with a sperm. — 72 hours: only one fruit could be investigated. The great majority of the embryo-sacs were fertilized, and in such embryo-sacs the polar nuclei were often united to a central nucleus; several had, however, polar nuclei. The division of the central nucleus (meta-, ana- and telo-phase) was observed only three times (a number of sections had been lost). —  $7 \times 24$  hours: two ovaries had probably only enlarged (that is fertilized) ovules, two had many not enlarged ovules. The latter were unfertilized, the former had two or mostly four endosperm nuclei. —  $8 \times 24$  hours: in three fruits most ovules, in one about half the number of ovules, in one no ovule was enlarged. The fruit last mentioned was very small. Enlarged ovules had four or eight endosperm nuclei, or four nuclei dividing. —  $12 \times 24$  hours: in one fruit less than half the number of the ovules were enlarged, in other fruits the great majority were enlarged. Enlarged ovules had in most cases eight, rarely four or sixteen, once only two endosperm nuclei. — A varying number of mitoses could be observed in the somatic tissue of the developing ovules in all fixations.

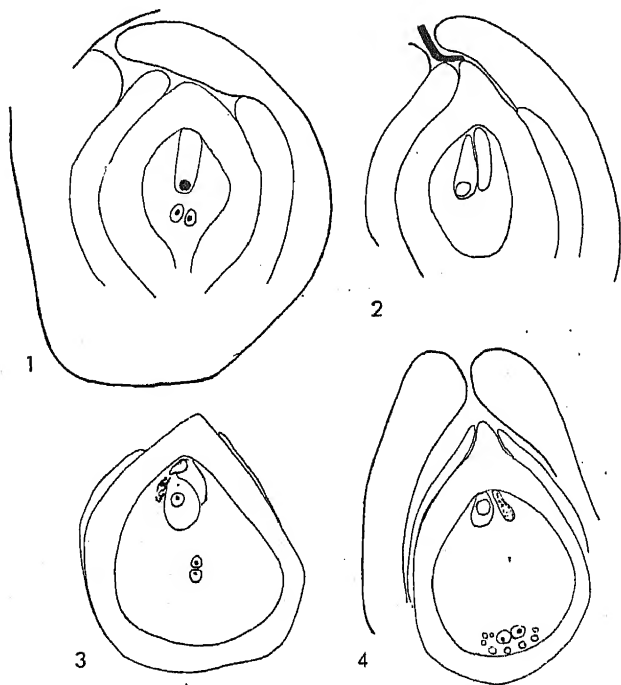
*Selfing of 3* (incompatible pollination). — 10 hours: in each of seven ovaries investigated only a few ovules were fertilized. — 25 hours: most embryo-sacs were fertilized, polar nuclei were sometimes of different size, indicating fertilization. — 48 hours: in one ovary were 31 fertilized, 3 unfertilized, in one 30 fertilized, 1 unfertilized, in one 13 fertilized, 24 unfertilized embryo-sacs. The polar nuclei were moving towards the chalaza. — 72 hours: four fruits were investigated, as a rule the embryo-sacs were fertilized. Only rarely a central nucleus was formed. The polar nuclei in fertilized embryo-sacs were as a rule near the chalaza and showed signs of being fertilized. Sometimes the pollen tube had disorganized a synergid, the egg-cell and polar nuclei were, however, not fertilized and no sperms were observed. Two embryo-sacs showed the division of the central nucleus, and two contained two endosperm nuclei. — Mitoses were rare in the ovules after 72 hours, but numerous after 48 hours.

$3 \times 5$  and  $5 \times 3$  (compatible pollinations). — 10 hours: in  $3 \times 5$  one ovary contained only fertilized embryo-sacs, one only unfertilized, while a third had about half the number of its embryo-sacs fertilized. Similar conditions were shown by  $5 \times 3$ . — 96 hours: in  $3 \times 5$  three fruits were relatively large and had most ovules fertilized, one fruit was rather small and had only about half the number of ovules fertilized, in another small fruit all ovules were unfertilized. Fertilized embryo-sacs had in most cases two endosperm nuclei, in some cases the division of the central nucleus was observed, the central nucleus was rarely undivided. In  $5 \times 3$  fertilized embryo-sacs had two or four endosperm nuclei, in one case, however, polar nuclei. —  $7 \times 24$  hours: in  $3 \times 5$  the enlarged ovules in most cases had four (sometimes two or eight) endosperm nuclei, in  $5 \times 3$  the most common number of endosperm nuclei was eight. — Mitoses in the fertilized ovules of some fruits were common, in other fruits more rare.

$2 \times 3$  and  $3 \times 2$  (probably compatible pollinations). — 96 hours ( $3 \times 2$ ): in one ovary most ovules were fertilized, in one only few were fertilized, in one about half the number were fertilized. Fertilized ovules had two or four endosperm nuclei. —  $7 \times 24$  hours ( $2 \times 3$ ): enlarged ovules had in most cases four, sometimes only two, endosperm nuclei (the occurrence of eight was doubtful). —  $7 \times 24$  hours, a wilted cherelle ( $2 \times 3$ ): about half the number of ovules were enlarged with in most cases four endosperm nuclei, the egg-cells were indistinct. The inner integument had degenerated in the unfertilized ovules, but this was also the case

in unfertilized ovules of the non-wilted cherule. —  $10 \times 24$  hours ( $2 \times 3$ ): enlarged ovules have in most cases eight endosperm nuclei. —  $8 \times 24$  hours ( $3 \times 2$ ): most ovules were enlarged. They had eight or more rarely four endosperm nuclei. A few of the ovules that had not been enlarged showed indication of a fertilization of the embryo-sac, however, their polar nuclei were not divided (Fig. 4). —  $12 \times 24$  hours ( $3 \times 2$ ): some ovules were degenerated. Enlarged ovules had mostly eight endosperm nuclei.

$3 \times 4$  and  $4 \times 3$  (probably incompatible pollinations). — 72 hours ( $3 \times 4$ ):



Figs. 1—4, ovules of *Theobroma cacao*. — 1: 5 selfed, 48 hours after pollination. — 2:  $3 \times 5$ , 25 hours after pollination, pollen tube in the micropylar canal. — 3:  $4 \times 3$ , 96 hours after pollination, the embryo-sac was probably not fertilized, the inner integument is collapsed; the outer integument is not figured. — 4:  $2 \times 3$ , 8 days after pollination, the pollen tube had penetrated into the embryo-sac but the polar nuclei are probably not fertilized; they are still surrounded by starch. —  $\times 230$ .

in the ovary investigated most embryo-sacs were fertilized. They had polar nuclei or a central nucleus, which often were not near the chalaza, rarely two endosperm nuclei. —  $4 \times 24$  hours, a dropped flower ( $4 \times 3$ ): one fruit, about half the number of embryo-sacs were fertilized. Such embryo-sacs had two, but in some cases four, endosperm nuclei (Fig. 14). In several embryo-sacs having a disorganized synergid polar nuclei had not been fertilized. — Mitoses were rather common in the inner integument after 72 hours, but could very rarely be observed in the dropped flower. In the latter, unfertilized ovules were smaller than fertilized and their inner integu-

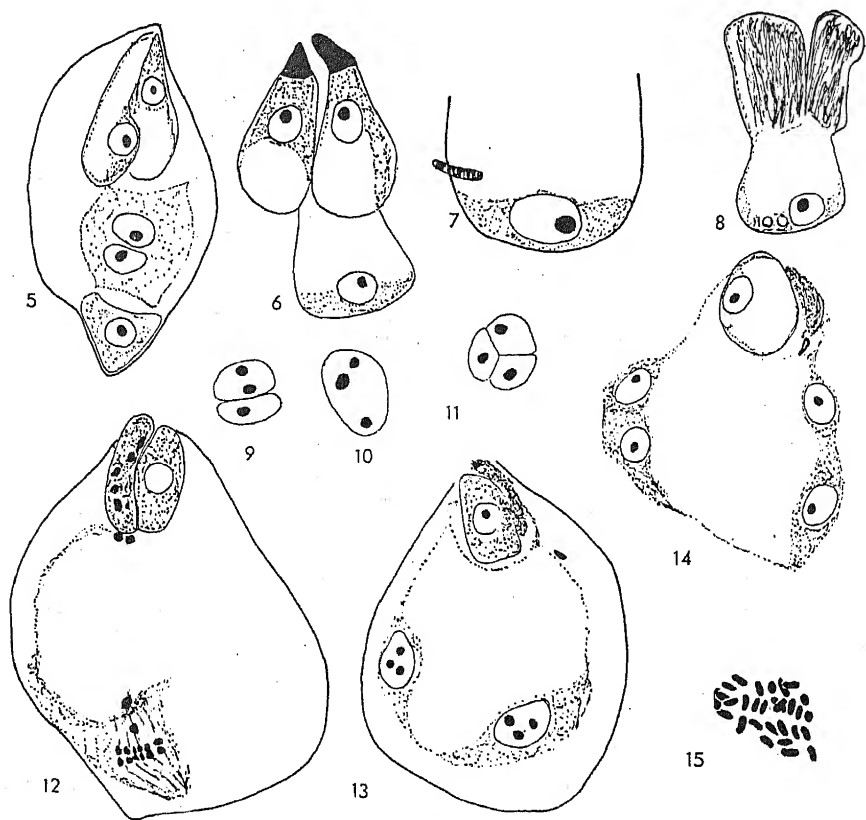
ment was often degenerated (Fig. 3). The egg-cell was distinct in most embryo-sacs of the dropped ovary.

From the observations made a general description of the seed development, which in some points supplements the papers of CHEESMAN and COPE, may deserve publication. The outer integument is higher than the inner integument; it is not, however, developed on the funicular side of the ovule. The inner integument has its strongest development on the funicular side, on the abaxial side of the ovule its growth is arrested before it reaches the apical part of the nucellus. Thus the micropylar canal, which is directed laterally towards the placenta, is on the funicular side limited by the inner integument, while the outer integument is in contact with the apical part of the nucellus (Fig. 1), the zone of contact sometimes being rather broad (Fig. 2). The pollen tube grows through the micropylar canal to the apex of the nucellus and then through the nucellar layers to the embryo-sac. The tube was more easily observed at the entrance of the micropylar canal or in the nucellus in 3 or  $3 \times 5$  than in 5 owing to the fact that it was thicker here (Fig. 2).

Only very rarely was the embryo-sac aborted. Organized embryo-sacs had a very large egg-apparatus (Fig. 5). The synergids are rather variable; the basal part of the synergid cells is sometimes a rather long cone, but in other embryo-sacs it is broad with conspicuous synergidal lists. Sometimes each synergid had a cap (Fig. 6); in slides stained with light-green the caps were green; they are thus probably made of cellulosa, as was expected. After the pollen tube has entered the embryo-sac one synergid is disorganized. The egg-cell is long and poor in cytoplasm. Fertilized egg-cells undergo certain changes, they become shortened, are relatively richer in cytoplasm and their nucleus is larger. They enter, as has been shown by CHEESMAN, into a long resting stage: after 12 days no egg-cell was divided in the Clementina material. The polar nuclei are in the vicinity of the egg-apparatus, after fertilization they move to a position near the chalaza, where the first division of the endosperm occurs (Fig. 12). In 5 they had more often than in 3 reached the chalaza after 48 hours. If fertilization of the embryo-sac fails, they as a rule remain in their original position, but are perhaps able to move later: a week after pollination they have sometimes been observed near the chalaza in embryo-sacs that were probably unfertilized. The antipodal cells of the embryo-sac are not particularly small but are ephemeral. The starch grains in the embryo-sac have been described and figured by CHEESMAN and COPE. They are often clustered

on the polar nuclei and follow these to the chalaza. After 72 hours they tend to be more dispersed, diminish in number and have often disappeared after 96 hours. In unfertilized embryo-sacs they remain longer.

CHEESMAN and COPE have pictured a small male nucleus in contact with the egg nucleus. CHEESMAN also figures a small male nucleus in



Figs. 5—15, *Theobroma cacao*. — 5: embryo-sac of 5; one synergid and two antipodals are not figured.  $\times 760$ . — 6: egg-apparatus of 5, the synergids have caps.  $\times 920$ . — 7:  $3 \times 5$ , 25 hours after pollination, sperm on the side of the egg-cell.  $\times 1900$ . — 8: 5 selfed, 24 hours after pollination; two male nuclei in the egg-cell.  $\times 920$ . — 9: 5 selfed, 72 hours after pollination; polar nuclei, one of them fertilized.  $\times 760$ . — 10: 5 selfed, 72 hours after pollination; central nucleus.  $\times 760$ . — 11: 3 selfed, 48 hours after pollination; polar nuclei and male nucleus.  $\times 760$ . — 12: 3 selfed, 72 hours after pollination; the first division of the endosperm.  $\times 760$ . — 13: 3 selfed, 72 hours after pollination; the embryo-sac has two endosperm nuclei.  $\times 760$ . — 14:  $4 \times 3$ , 96 hours after pollination; four endosperm nuclei.  $\times 760$ . — 15: nuclear plate — the endosperm divisions; the number of chromosomes is (about) 30.  $\times 1900$ .

contact with the two polar nuclei. I have found observations on fertilization difficult. The male nuclei seem to change form and size when introduced into the embryo-sac. Fig. 7 shows a spindle-like chromophilic sperm from the vicinity of the egg-cell. Later the sperm seems to have taken the appearance of an ordinary nucleus as in the figures of CHEESMAN and COPE; the egg-cell in Fig. 8 seemed to contain two such nuclei. Fig. 11 indicates that the male nucleus may approach the size of a polar nucleus. However, in the Clementina material triple fusion only rarely occurs. In fertilized embryo-sacs the polar nuclei were of different size and one of them had two nucleoli (Fig. 9). Thus the male nucleus in most cases fertilizes one of the polar nuclei; later the polar nuclei unite, forming a central nucleus having several nucleoli (Fig. 10). A central nucleus was only observed after 72 hours, and thus is formed immediately before the first division of the endosperm. In only a minority of the embryo-sacs of 5 did this division occur at 72 hours, and thus it is distinctly later than in the compatible combination studied by COPE and about as late as in the material of CHEESMAN. After 96 hours the fertilized embryo-sacs as a rule had two (or more rarely four) endosperm nuclei; undivided polar nuclei were then rare. This was also the case in the probably incompatible combination  $4 \times 3$ , and here the more sluggish development found by COPE after incompatible pollination perhaps did not occur. The latest fixation made of the incompatible 3 was after 72 hours. In some ovules the first division in the endosperm was observed or had occurred, but, as many more ovules could be studied of 3 than of 5 while the number of observed divisions was about the same in the two cases, it seems that the division is retarded in the incompatible combination. And it is a fact that a central nucleus could only seldom be observed in 3, the polar nuclei being rarely united after 72 hours. Thus selfing of 3 seems to result in a belated fertilization and a retarded nuclear activity in the fertilized embryo-sac.

The first endosperm nuclei are relatively large and have several nucleoli. The size of the endosperm nuclei diminishes in the following divisions. The chromosome number of the endosperm was as expected 30 (Fig. 15). The rate of development of the endosperm is slow. There seemed to be differences of this rate in the two reciprocal crosses with 3 and 5, but the investigated material is obviously too small to allow of any certain conclusions in this respect. In some ovules of a cherelle the rate of development of the endosperm was retarded. There are also

differences in the size of the embryo-sacs in the same cherelle, some of them being conspicuously broader or smaller than the majority.

The inner integument has two or three layers at the time of fertilization. In all fixations of 5 one observes cell-divisions in the inner integument and nucellus of the fertilized ovules; if fertilization fails the divisions cease. Many mitotic spindles have a radial orientation and therefore the bulk of the nucellus and the number of layers of the inner integument increase, the latter to 4—5 after a week and 5—6 after 12 days. After the probably compatible crosses between 2 and 3, mitotic divisions seemed as a rule rarer than in 5, and this is confirmed by the inner integument having only 4—5 layers after 10 days, 4 layers after a week. After a week the inner integuments of  $3 \times 5$  had 4 layers, those of  $5 \times 3$ , 4—5 layers. The incompatible selfing of 3 had mitosis in many ovules after 48 hours, but after 72 hours only few of the fertilized ovules had one or two divisions in the inner integument and the nucellus. In the probably incompatible combination  $3 \times 4$ , however, cell-divisions were numerous in many ovules after 72 hours, but very few divisions could be observed after 96 hours (in a dropped flower).

In all cherelles investigated there was at least some unfertilized ovules. Incompatible pollination seems to result in belated fertilizations, but after 48 hours the great majority of the ovules may be fertilized; after compatible pollinations, too, as a rule not all ovules are fertilized. The unfertilized ovules of the cacao seem to merit a description. 96 hours after pollination ovules with unfertilized embryo-sacs are distinctly smaller than fertilized ovules in the same cherelle owing to the earlier cessation of cell-division. The embryo-sac has, however, enlarged. It is quite as broad as the fertilized embryo-sacs with endosperm nuclei but shorter. During the widening of the unfertilized embryo-sac several nucellus layers are destroyed. The behaviour of starch grains and polar nuclei have been described above: the cells of the egg-apparatus soon become poor in cytoplasm, and are often indistinct. A degeneration of the somatic tissue occurs and this degeneration always begins with the inner integument. As early as 96 hours after pollination in some or most unfertilized ovules the cells of the inner integument have collapsed, and after a week the inner integument has collapsed in all unfertilized ovules. The remaining nucellus layers also collapse and, as the inner integument is mostly close to the nucellus layers, the unfertilized ovules after a week have a peculiar hollow appearance like an empty shell (Fig. 3). Then the ovules dry and the lumen of the embryo-sac shrinks; after 10 or 12

days almost all unfertilized ovules have dried. A similar appearance is assumed by ovules where a pollen tube had disorganized a synergid but the polar nuclei could not divide, probably due to failure of fertilization (Fig. 4).

*Conclusions.* — Embryological studies may help to an understanding of the different sterility in cacao: the early drop of many ovaries, the cherelle wilting and the aborted seeds in such pods as reach maturity. A special problem is offered by the incompatibility. The early drop after compatible crossings is probably mostly a result of failures of pollination. POUND found that unpollinated flowers remain on the tree 48 hours at the highest, owing to the function of an abscission mechanism. The material here investigated had been hand-pollinated and therefore a number of pollen grains probably sufficient to fertilize all ovules in the ovaries had been brought to the stigmas. It is, however, a fact that as a rule a variable number of the ovules in these hand-pollinated flowers were unfertilized. In certain extreme cases no ovule was fertilized, and such ovaries could remain on the tree a week. This is perhaps an indication that pollination *per se*, that is pollination not followed by fertilization, tends to retard the function of the abscission mechanism. Cherelles with no fertilized ovules were much smaller than normal cherelles of the same age; an intermediate size had cherelles where about half the number of ovules were fertilized. That the abortion of seeds in the pods to a large extent depends on failures of fertilizations is quite clear; in a cherelle 12 days old no doubt half the ovules could be unfertilized. In some cases the ovule was fertilized but development did not start. There were also a certain though low number of fertilized ovules that were unusually small and had a narrow embryo-sac with an abnormally low number of endosperm nuclei. Such ovules probably degenerate later, having too low a rate of development, and may thus increase the seed sterility. A fourth cause of seed abortion may perhaps be degeneration of the egg-cell or the embryo. Such abortions could not be studied and it is unknown whether they occur in any appreciable frequency.

Concerning the question of cherelle wilting, it has already been mentioned that POUND found the number of good ovules to be about the same in good and wilting cherelles. However, an embryological investigation has not been made; many ovules in the wilting cherelles are perhaps abnormal. Professor MÜNTZING was only able to make fixations during 12 days, and thus the cherelle wilting could not be studied here. BRINK and COOPER (1941) have shown (their paper has also been cited

by MÜNTZING) that in certain species hybrids there are a slow development of the endosperm and an excessive growth of the somatic tissue of the ovule (integument or nucellus), leading to starvation and death of the embryo. This kind of sterility they call somatoplastic sterility, and they consider the abnormality of the somatic tissue to be a cause of the slow development of the endosperm, and this in its turn leads to the abortion of the embryo and the seed. After a review of the surprisingly meagre literature on the embryology of dropped orchard fruits they express the opinion that the so-called June drop of orchard fruits is a result of abortion of seeds, this seed abortion being caused by somatoplastic sterility. It may be mentioned that LUCKWILL (1946), in apple seeds from three to ten weeks old, found a fruit setting hormone. The fructigenic activity of the seeds expires about 10 weeks after the petal fall, that is at the time of the June drop.

It is tempting to compare the cherelle wilting of cacao with the June drop of orchard fruits, somatoplastic sterility perhaps causing a weak development of the seeds and thus an insufficient production of a fruit setting hormone. However, one feature of somatoplastic sterility, excessive development of nucellus and integument, one would hardly expect to find here. CHEESMAN showed that there is »a considerable development of the nucellus, forming perisperm, whilst up to the time of wall-formation the endosperm is a very thin layer lining the embryo-sac. From this point onwards the endosperm increases more rapidly, and the perisperm is soon reduced in proportion». The first wall-formation in the endosperm was observed by CHEESMAN in fruits about fifty days old. As normally the development of nucellus is strong and of endosperm weak, it seems unlikely that excessive development of nucellus can be a cause of seed abortion in cacao. However, the developing endosperm may perhaps sometimes have inherent weakness leading to abortion, the endosperm not being able to reduce the perisperm. An embryological investigation of the state of the seeds in good and wilting cherelles is, however, needed.

The incompatibility in cacao is not a result of failure of fertilization, as has been shown by COPE, who, however, found the development in the embryo-sac to be more sluggish after incompatible pollinations. The delayed and slow development of the embryo-sac after incompatible pollinations is responsible for the failure to set fruit and the abscission. »Where, as in compatibly pollinated ovaries, activity of the contents of the embryo-sac is at a high level of intensity, then the turgor abscission mechanism does not operate.» COPE had investigated

cacao from Trinidad. POSNETTE concludes concerning the self-incompatibility in Amazonas cacao (here crosses between incompatibles are compatible): »It is tentatively suggested that the failure to inhibit the maternal mechanism of flower-shedding occurs when the pollen tube carries the same incompatibility gene as the maternal tissue» (1945). Of the two incompatible combinations investigated here,  $3 \times 3$  and  $3 \times 4$ , the former showed a slower development in the fertilized embryo-sacs than compatible combinations, though the difference does not seem to be as great as that found by COPE. I could not observe that »some sacs were completely devoid of nuclear content, although starch persisted» (COPE, l. c.); such a state of things having only been observed by me in older unfertilized embryo-sacs. There was less evidence of sluggish activity in fertilized embryo-sacs of the combinations  $4 \times 3$ ; as a rule fertilized ovules had two or four endosperm nuclei after 96 hours.

The observations made here indicate that after incompatible pollinations fertilized ovules soon show no divisions in nucellus and the inner integument. After 72 hours only a few ovules had some cell-divisions in  $3 \times 3$ , and in  $3 \times 4$  they had ceased after 96 hours even if the embryo-sac of the ovule had endosperm nuclei and therefore had shown normal activity. If fertilization fails, the cell-divisions in the somatic tissue of the ovule stop and the cells of the inner integument soon collapse, the degeneration being first manifest in this tissue. Fertilization starts a new nuclear activity in the somatic tissue of the ovule, mitoses in nucellus and inner integument soon becoming numerous, but it seems that the stimulus to new nuclear activity is weak after an incompatible fertilization. This cessation of mitosis was evident in all ovules of the ovary that had been pollinated incompatibly even if they had an embryo-sac with four endosperm nuclei, and thus perhaps the fertilization (of the polar nuclei?) has a direct influence on the somatic tissue. After incompatible fertilizations this influence is perhaps too weak; there is soon no nuclear activity in the somatic tissue and therefore the function of the abscission mechanism is not stopped.

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# DISTURBANCES IN MICROSPORE CYTOLOGY OF ANTHOXANTHUM

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IN 1935 GEITLER showed that the microspore is a polarized cell in which the axis of the division of the nucleus of the pollen grain is constant and characteristic of each species or genus.

The development of the microspore in *Tradescantia* has been carefully described by SAX and EDMONDS (1933). From the description of these authors we can recognize three steps in the development of the pollen grain before the second pollen mitosis: firstly, the movement of the nucleus to a special position accompanied by changes in the cytoplasm and movements of the vacuole; secondly, the orientated division; and, thirdly, the differentiation of the two new nuclei. The second pollen division involving only the generative nucleus follows in the pollen tube during the germination of the grain.

In *Anthoxanthum*, as in other grasses, the first division of the pollen grain has a characteristically oriented and asymmetric spindle and it gives rise to two nuclei of different shape and appearance: one darkly stained that originates near the cell-wall, and another faintly stained larger and more rounded that originates in the middle of the cell. The second division also takes place inside the pollen grain, it consists in the division of the more stained nucleus called the generative, while the other, the vegetative, becomes more and more pale, and remains a resting nucleus. In this division of the generative nucleus, the chromosomes are much contracted, and after telophase the two daughter nuclei start to assume spindle shape.

In the material of wild origin of *Anthoxanthum aristatum* BOISS., with B chromosomes, kindly ceded by Dr. G. ÖSTERGREN (ÖSTERGREN, 1947), there were found some disturbances in this process.

It was observed that in 7 plants with different numbers of B chromosomes the disturbances were as listed in Table 1.

*Non-orientation, weak or non-differentiation of the two nuclei.* — In all the plants, besides the pollen grains with normal orientation and differentiation, there could be found as an occasional intermixture, varying from anther to anther, such in which the two nuclei were un-

TABLE 1. *Disturbances in the microspores of A. aristatum.*

Reference number	Number of B chromosomes	Disturbances observed		
		Non-orientation, weak or non-differentiation of the 2 nuclei	Supernumerary mitosis	Chromatin drops
1	1			
2	1	»	—	—
3	1	»	—	—
4	2	»	—	—
5	2	»	—	—
6	2	»	—	—
7	4	»	—	—

distinguishable from one another or where they presented all degrees of transition between complete differentiation and no kind of differentiation (Figs. 19 and 20). This non-differentiation was accompanied by an absence of orientation and the nuclear development was in most cases displaced in the direction of a weaker staining or less commonly in the direction of a stronger staining, that is to say, the two nuclei approximated the appearance of a vegetative nucleus or in a few cases (plant 2) that of a generative nucleus.

In plants 2 and 7 there could also be found, besides these non-differentiated grains, other abnormal pollen grains with degenerating nuclei and empty pollen grains. These last ones have been considered as the final result of the degeneration of those non-differentiated pollen grains which have been unable to proceed to division and life. This view was supported by the fact that in the same anther, besides non-differentiated pollen grains, there were many pollen grains with three well differentiated nuclei (with two sperm nuclei already of spindle shape). This shows that these non-differentiated nuclei really could not reach division, or were much delayed in attaining it.

In another plant (plant 1) non-differentiation was not only followed by degeneration but also by supernumerary mitosis; the two nuclei were able to divide and they sometimes reached the stage of 4 nuclei.

*Supernumerary mitosis, chromosome contraction and spindle disturbances.* — In plant 1 it was found that the pollen grains in some cases develop with a normal differentiation and orientation of their nuclei, which then also divide normally, but in other cases there results

a non-orientation and weak differentiation connected with division of one or both the two nuclei formed after the first division. It has been possible to recognize a complete series of transitions between, on one hand, the division of only the generative nucleus and non-division of the vegetative nucleus (the normal case) and, on the other hand, the complete division of the vegetative nucleus without the division of the generative.

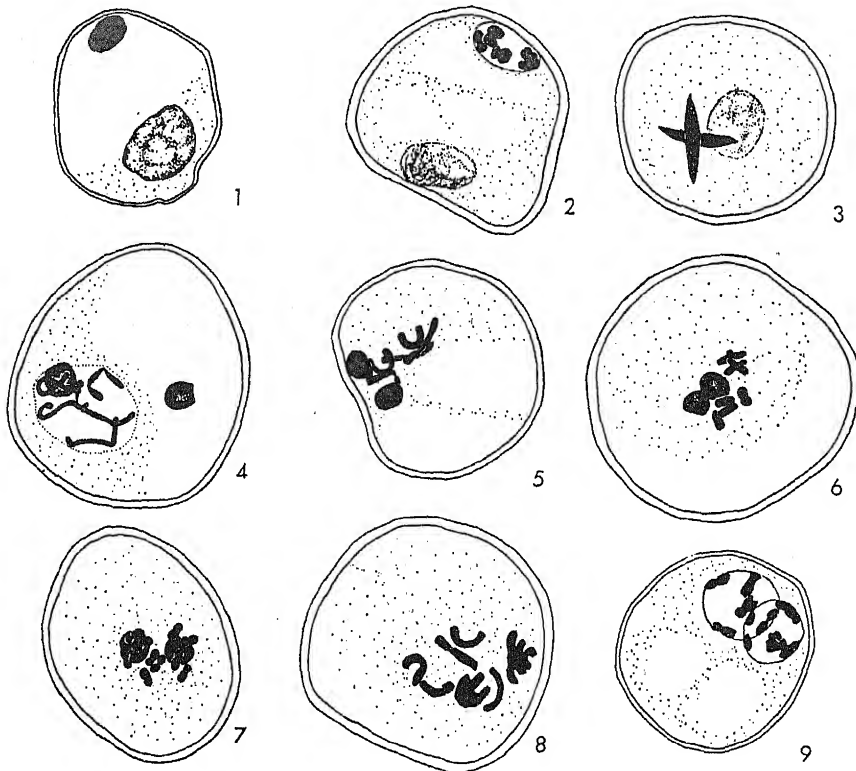
Looking at Figs. 1 to 3 we can see the normal case (case 1) in which the vegetative nucleus remains palely stained and the generative (Fig. 1) begins to divide (Fig. 2), giving origin to the 2 spindle-shaped sperm nuclei (Fig. 3). The chromosomes appear (Fig. 2) already contracted.

In the second case, after the normal division of the generative nucleus, the vegetative begins to divide. In Fig. 4 a prophase of the vegetative nucleus with the five chromosomes is seen between two typical sperm nuclei; in Fig. 5 a normal metaphase plate in the middle of two sperm nuclei; in Fig. 6 a metaphase in which the chromosomes are just divided and much contracted, with the appearance of a colchicine-mitosis; in Fig. 7 the case of an abnormal anaphase in which the pairs of chromosomes can still be seen, coming most probably from an abnormal metaphase like that of Fig. 6. Figures very similar to these with c-mitotic appearance and extra contraction have been recorded by BARBER (1941) after heat-treatment in the pollen grains of *Uvularia*, and SAX (1937 b) has also found contraction of the chromosomes in *Tradescantia* (a triploid plant).

The third case (Fig. 8) is considered as being most probably the result of the division of the generative nucleus immediately followed by the division of the vegetative, which is developing a metaphase plate between the two anaphase groups of the generative nucleus.

In case four (Figs. 9 and 10) a complete synchronization of the two nuclei is found and it is only possible to guess which is the vegetative and which is the generative nucleus by the number of chromosomes, but in all other respects they are undistinguishable.

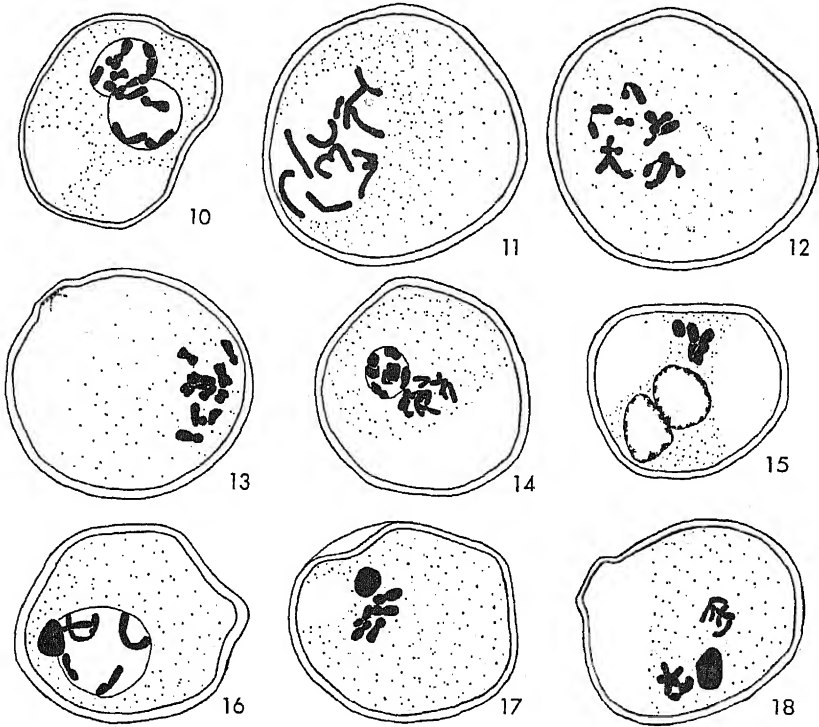
In *Anthoxanthum* (ÖSTERGREN, l. c.) as in rye (MÜNTZING, 1946) the B chromosomes perform a non-disjunction at the first pollen mitosis and are included in the generative nucleus, the vegetative nucleus being without B chromosomes. As, in *A. aristatum*, the number of chromosomes is  $2n=10$  and plant No. 1 has 1 B chromosome, when the pollen grain gets 1 B chromosome the vegetative nucleus will get only 5 chromosomes, and the generative will get 7 (Figs. 9, 10, 14), but if the pollen grain does not get any B chromosome each nucleus will have 5



Figs. 1—18. Series in the course of division of the pollen nuclei from the normal case to the division of the vegetative nucleus without division of the generative. — Figs. 1—3, case 1, normal case. — Figs. 4—7, case 2, division of the vegetative nucleus after the division of the generative. — Fig. 8, case 3, probable division of the vegetative

chromosomes and they will be undistinguishable. It must be pointed out in this connection, however, that the utility of the B chromosomes in recognizing the vegetative and generative nucleus depends entirely on the truth of the assumption that the distribution mechanism of the B functions normally, an assumption which may not hold under these abnormal conditions.

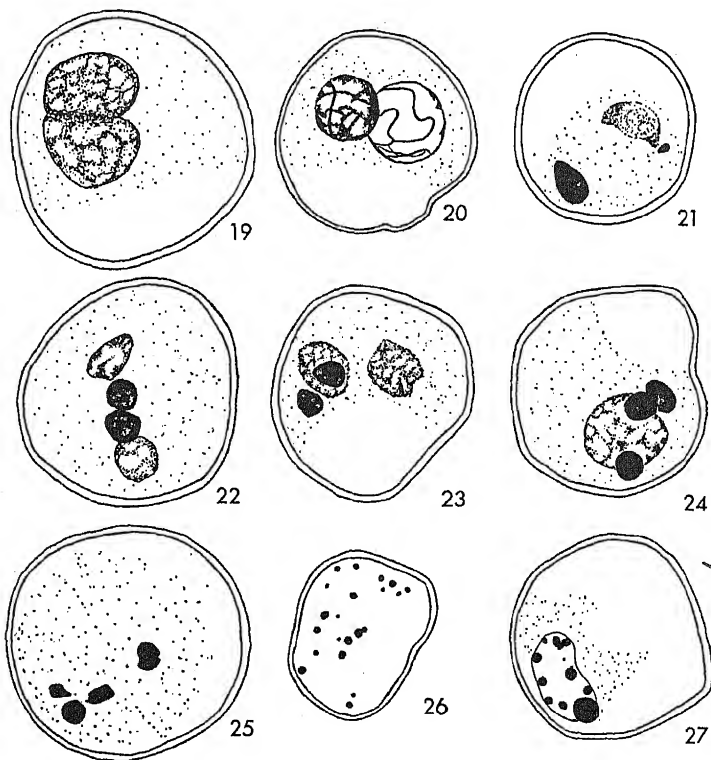
Through the synchronization of the division of the two nuclei they give origin to a common metaphase (Figs. 11, 12 and 13), which shows non-orientation of the chromosomes in the metaphase plate and sometimes strong contraction of the chromosomes (Fig. 13) like the prophase chromosomes of Figs. 9 and 10. What the destiny is of these two simultaneous mitoses could not be discovered with exactitude. We will come later to this point. A similar case of simultaneous but not syn-



nucleus when the generative is still at anaphase. — Figs. 9–13, case 4, synchronized division of the two nuclei and common metaphase. — Figs. 14 and 15, case 5, division of the generative nucleus after that of the vegetative. — Figs. 16–18, case 6, division of the vegetative nucleus without division of the generative. —  $\times 1290$ .

chronized mitoses has been found by DARLINGTON and JANAKI-AMMAL (1945) in *Nicandra*, where the two nuclei divide at the same time but with a difference in phase. DARLINGTON and THOMAS (1941) have also found a common anaphase in *Sorghum* of two secondary central nuclei which have developed a third mitosis at their previous fusion, although this case differs from the *Anthoxanthum* case reported here, because in *Sorghum* the two nuclei are of vegetative nature while in *Anthoxanthum* one may be a generative and the other a vegetative nucleus, at any rate in many cases.

Case five shows (Fig. 14) the division of the generative nucleus (7 chromosomes) still at prophase when the vegetative is already at anaphase, in Fig. 15 the two vegetative nuclei were formed and the generative nucleus is reaching metaphase (note contraction of the chromosomes).



Figs. 19 and 20. Pollen grain with non-differentiated nuclei (Fig. 19) or weak differentiated nuclei (Fig. 20). — Fig. 21, pollen grain with chromatin drop. — Figs. 22—24, pollen grains with 4 nuclei considered as the result of supernumerary mitosis. — Figs. 25—27, pollen grains with a chromatin constitution considered, probably, as the result of supernumerary mitosis. —  $\times 1290$ .

Case six shows the non-division of the generative nucleus accompanied by the division of the vegetative. The generative nucleus is, as usual, recognized by its smaller size and stronger staining ability. In Fig. 16 it is at prophase, in Fig. 17 at metaphase (note contraction and irregular distribution), and in Fig. 18 at anaphase.

These six cases show a complete seriation in the asynchronism of the division of generative and vegetative nuclei, which rises from the normal case of the division of the generative nucleus without division of the vegetative, passing through the complete synchronization of the division of the two nuclei to the division of the vegetative nucleus without any division of the generative.

The result of these supernumerary mitoses could not be definitely

ascertained. However, some observations that have been brought together in Figs. 22 to 27 show that in some cases the two divisions give origin to four nuclei and that of these four nuclei 2 are of a generative type and 2 of a vegetative type (Figs. 22 and 23), while in other cases 4 nuclei are formed which are not so well marked in type, for instance Fig. 24, which shows a pollen grain with 4 nuclei, 3 of which are of a generative type and one of a vegetative type, and also a case with 4 nuclei (2 fused) with a vegetative appearance, and another (Fig. 25) with 4 nuclei, two of which have not a regular nuclear shape; we consider them to be most probably the result of a common metaphase. In other cases it is possible to find pollen grains full of drops of a chromatin nature (Fig. 26) which most likely are another result of a common metaphase. In another case these chromatin drops appear in a prophase nucleus that seems to have degenerated before proceeding any further.

*Chromatin drops.* — Only in plant 1 has there been found a large quantity of pollen grains with chromatin drops in the cytoplasm (Fig. 21). In other plants only one or two pollen grains were found that contained them.

These drops appear in pollen grains with one, two and three nuclei but especially in uninucleate pollen grains, less often in binucleate ones and only a few in trinucleate pollen grains.

Contrary to the data obtained by MÜNTZING (l. c.) in rye, the frequency of chromatin drops in the present case is higher in uninucleate pollen grains than in binucleate ones. This shows that most probably these drops have their origin from the chromosomes of the normal complement and not from the B's, as most likely was the case in rye. Because, if the chromatin drops were from the B's, owing to the fact that the B's delay division (ÖSTERGREN, l. c.) those pollen grains which have chromatin drops will not get the B's in their nucleus and hence on an average they will divide more quickly than the others. But, as we can see from the data of Table 2, this is not what happens, the pollen grains that have chromatin drops are not hastened, but are on the contrary delayed in their division. Thus the chromatin drops most probably have their origin in the chromosomes of the normal complement. The elimination of a normal chromosome from the nucleus is most likely, either strongly to retard mitosis of that pollen grain or completely to inhibit it. No doubt, these drops indicate some asynapsis during meiosis in this plant. There is one more reason to show that

TABLE 2. *Relation between chromatin drops and division.*

Number of chromatin drops	Number of pollen grains with 1 nucleus	Number of pollen grains with 2 nuclei
0	24	20
1	82	13

they must derive from the normal complement; B's are not eliminated at meiosis, not even when unpaired (ÖSTERGREN, l. c.).

The analysis of these data indicates a significant difference, thus we are able to admit a positive correlation between chromatin drops and delay in division.

The presence of chromatin drops is not correlated to the presence of B's in another respect, that is to say, they appear only in plant 1 which has 1 B chromosome and not in other plants with the same number of B's or in plants with a higher number (2 or 4). It consequently seems that they most probably have their origin in an unbalanced genetic constitution of the plant, an unbalance to which the B most probably does not contribute at all or, mostly, to a minor extent.

## DISCUSSION.

*Relation between orientation and differentiation.* — Non-differentiation is known to occur in a low frequency (7 %) under normal conditions in *Tradescantia gigantea* (SAX, 1937 b), and can be produced by different agencies. SAX (1935) has been able to produce the lack of orientation and differentiation in the pollen grains of *Tradescantia* by cold as well as by heat-treatment, and has found that the degree of differentiation of the two nuclei is closely associated with the angle of division. Temperature seems to affect cytoplasmic movement though not so much nuclear division. In a single anther SAX has found all degrees of differentiation, including the normal condition. He has also observed that heat-treatment is able to induce non-differentiation in the microspores of *Pseudolarix amabilis*, but in this case a difference was found from the *Tradescantia* case, that is, the differentiation of the two nuclei was not directly dependent upon the spindle orientation but upon the position more or less close to the wall of one of the two nuclei. In this case differentiation seems to be regional, that is to say, to be associated with some regions of the cytoplasm of the pollen grain (in this case the wall). Thus, it seems that differentiation is connected neither ab-

solutely with orientation nor absolutely with region, but it is connected with cell architecture, which in one case is expressed in one way and in another case in another way, and this architecture is the result of synchronization of the cytoplasmic and nuclear activities.

KOLLER (1943) could also obtain a loss of differentiation by means of the action of X-rays upon the pollen grains of *Tradescantia*, in which case the lack of differentiation was positively correlated to the presence of micronuclei.

An observation showing that differentiation is not directly connected with orientation is that made by SAX and HUSTED (1936) in *Periploca septium*. The pollen grains of this species do not separate, remaining in tetrads, and the spindle of the microspore division may be in any plane, but »differentiation of generative and tube nuclei is complete, regardless of their orientation in the cell«.

Thus we are able to conclude that orientation is not always directly connected with differentiation, which shows that simultaneity does not necessarily imply connection. The two phenomena seem to depend more on the timing of the activities of the nucleus and cytoplasm.

*Relation between non-differentiation and supernumerary mitosis.* — The relation between non-differentiation and supernumerary mitosis seems to be of the same kind.

There are already some cases in which supernumerary mitosis has been recognized (Table 3). In normal conditions it has been reported by SAX (1935) in a plant of *Tradescantia* growing in a greenhouse, and in normal plants of *Sorghum* by DARLINGTON and THOMAS (l. c.).

Supernumerary mitosis has also been induced under experimental conditions. In *Hyacinthus*, after heat-treatment, DE MOL (1923) found pollen grains with 2 to 8 globular, non-differentiated nuclei. By the action of temperature in *Tradescantia* SAX (1935) obtained supernumerary mitosis, accompanied by lack of differentiation. In *Nicandra*, which species contains a peculiar isochromosome, DARLINGTON and JANAKI-AMMAL (l. c.) also found a second division of the vegetative nucleus attended by non-differentiation (in 25 % of the pollen grains of an anther from a single plant).

In the material of *Anthoxanthum* discussed here one plant showed supernumerary mitosis of the vegetative nucleus accompanied by non-differentiation, while the other six plants all had non-differentiation without supernumerary mitosis. Non-differentiation seems to be connected, though not directly, with supernumerary mitosis.

TABLE 3. *Supernumerary mitosis in microspores.*<sup>1</sup>

Normal conditions	Experimental conditions	In plants with unbalanced genetic constitution
<i>Tradescantia</i> , SAX (1935) <i>Sorghum</i> , DARLINGTON and THOMAS (1941)	<i>Hyacinthus</i> , DE MOL (1923) <i>Tradescantia</i> , SAX (1935)	<i>Tradescantia</i> (triploid), SAX (1937 b) <i>Amaryllis</i> (polyploid), UPCOTT (1939) <i>Sorghum</i> (B's), DARLINGTON and THOMAS (1941) <i>Nicandra</i> (isochromosomes), DARLINGTON and JANAKI-AMMAL (1945) <i>Anthoxanthum</i> (B's), the present paper

<sup>1</sup> The cases recorded by BEADLE (1931) and JOHANSSON (1944) are considered as supernumerary mitosis somewhat different from those listed here.

*Relation between supernumerary mitosis and unbalanced genetic constitution.* — DARLINGTON and THOMAS (l. c.) found in their material a relation between supernumerary mitosis and B chromosomes. In *Sorghum*, where they found supernumerary divisions of the vegetative nucleus, they observed that the extra divisions are stimulated by the presence of B's, having noted in all the 20 pollen grains studied in this respect that B chromosomes were present at the first extra pollen division. Anyhow, this is not exactly the case in *Anthoxanthum*, as can be seen from the presence of B's in the supernumerary mitosis of Figs. 9, 10 and 14 and their absence in Fig. 15.

These authors have also observed a variation in intensity of supernumerary divisions, conforming with a variation in the frequency of B's. By a glance at Table 1 it is possible to see that this is not the case in *Anthoxanthum*: the plants with the highest number of B's have no supernumerary mitosis, and of plants with the same number: one has and the others have not.

DARLINGTON and JANAKI-AMMAL (l. c.) found a second division of the vegetative nucleus in *Nicandra* with isochromosomes. In polyploid material as *Amaryllis Belladonna* (UPCOTT, 1939) and triploid *Tradescantia* (SAX, 1937 b) a second division of the vegetative nucleus has also been observed several times in *Amaryllis* and occasionally in *Tradescantia*.

This indicates that supernumerary mitosis may sometimes be correlated to a genetic unbalanced constitution, but we can see from the facts here reported from *Anthoxanthum* that the correlation is not absolute.

*Conclusions.* — If life is considered to be dependent upon a series of reactions which normally proceed at rates bearing a definite relation to one another, an idea pointed out by OSTERHOUT (1921), it is clear that a disturbance of these rate relations should have profound effects upon the organism.

Disturbances in such rate relations are known to have different origin and to lead sometimes to the same phenotypical result, as is known from GOLDSCHMIDT's works (GOLDSCHMIDT, 1938); and, as SAX (1937 a) reported, abnormalities induced by temperature treatment are similar to such induced by narcotics, osmotic pressure, X-rays, genetic factors, and other agencies. Most of these various agencies seem to induce abnormalities in cell and nuclear division by producing changes in the synchronization of nuclear and cytoplasmic activities.

The abnormalities reported in this work, viz. lack of orientation, non-differentiation, asynapsis (chromatin drops) and supernumerary mitosis, seem to be of this kind. They can have their origin in environmental conditions or in the unbalanced genetic constitution of the material. Thus they are most probably connected, though only because they are different expressions of the same cause — disturbances in timing.

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### SUMMARY.

In the pollen grains of *Anthoxanthum aristatum* BOISS. with B chromosomes, lack of orientation and non-differentiation of the nuclei have been found in 7 plants with from 1 to 4 B's.

A complete seriation between the division of the generative nucleus unaccompanied by that of the vegetative nucleus (the normal case) and a division of the vegetative nucleus unaccompanied by that of the generative has been observed. Synchronized mitoses of both generative and vegetative nuclei, giving rise to common metaphase, are presented.

A positive correlation between chromatin drops and delay in division is recognized. Their origin is attributed to chromosomes of the normal complement.

A discussion of the relation between these phenomena is developed; the conclusion is drawn that, most probably, they are different expressions of disturbances in timing relations.

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# THE RESISTANCE OF COLCHICUM AND BULBOCODIUM TO THE C-MITOTIC ACTION OF COLCHICINE

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## I. INTRODUCTION.

SINCE *Colchicum autumnale* in its tissues contains a considerable amount of colchicine — up to 1 % —, its own mitoses would be expected to be unaffected by this substance. In most higher plants the mitotic mechanism is switched over into so-called c-mitosis under influence of colchicine concentrations very much lower than those present in *Colchicum*. During c-mitosis any increase in cellular number is made impossible. Different authors have shown that pulp from crushed tissues of *Colchicum* may induce full c-mitosis in other plants. BLAKESLEE (1939) was the first one to point out the resistance of *Colchicum* to colchicine. He found that the c-tumour reaction could not be obtained in *Colchicum* by colchicine treatment. The behaviour of the root mitosis in *Colchicum* during long treatment with 1 % colchicine solution was studied by LEVAN (1940), who found no tendencies to c-mitosis. In 1943 MAIROLD repeated the testing of *Colchicum* with concentrations of crystalline colchicine up to 1 % in temperatures reaching + 30 centigrades. (In the present paper we always understand by »crystalline colchicine» the chloroform-containing crystalline form of the alkaloid.) MAIROLD found an absolute resistance as far as the c-tumour reaction was concerned: »So wurde auf diese Weise erneut die weitgehende Immunität der *Colchicum*-Wurzeln gegenüber Colchicin bewiesen, selbst unter Bedingungen, bei denen das Colchicin sonst besonders intensiv wirkt» (l. c., p. 494).

An American author, CORNMAN (1941, 1942), has re-tested the reaction of *Colchicum* to colchicine and arrives at another result than the above workers. He employed a very delicate method: the upper ends of free *Colchicum* roots were embedded in agar with 5 % glucose, while the lower ends were dipped into the solutions to be tested. Mitoses could still be found in the severed roots after a treatment of 8 1/2 hours. According to CORNMAN, 5 % colchicine for 4 hours give complete spindle

destruction in *Colchicum byzantinum*, the corresponding values for *Colchicum autumnale* were a 10 % solution for 6 hours. The latter species was thus more resistant than the former one, the resistance of neither of them being absolute.

As a continuation of our work on the action of c-mitotic substances we decided to re-investigate the resistance of *Colchicum*. Among the above authors only MAIROLD gave particulars as to what kind of colchicine she used, viz. MERCK's colchicine cryst. with 12,5 % chloroform. It may be communicated here that LEVAN (1940) in his testing of *Colchicum* used SANDOZ' colchicine amorph. Since it has been shown that pure chloroform may induce typical c-mitosis in *Allium* (ÖSTERGREN, 1944; STEINEGGER and LEVAN, 1947 b), it was close at hand to direct attention to the chloroform content of the crystalline colchicine. In the earlier paper just mentioned we studied the influence of the chloroform contained in crystalline colchicine ( $C_{22}H_{25}NO_6 \cdot \frac{1}{2}CHCl_3$ ) on a plant not resistant to colchicine, viz. *Allium Cepa*. We arrived at the conclusion that the content of chloroform present in this colchicine is of no consequence in the low concentrations at which the threshold value for c-mitosis is situated in *Allium*, i. e. about 0,004 % colchicine. In the present work two plant species resistant to colchicine are studied. At such strong concentrations of colchicine as have been tested in *Colchicum*, crystalline colchicine would, however, hold a chloroform content which might well be expected to give c-mitosis in this plant, provided it is not resistant to chloroform.

MAIROLD, using 1 % of crystalline colchicine, obtained a negative result as regards the c-tumour reaction. This fact, however, cannot be considered a conclusive evidence that *Colchicum* is absolutely resistant, since c-tumours are hard to induce by chloroform owing to the toxicity of this substance. The mitoses were not studied by MAIROLD. In the case of CORNMAN it is not recorded what quality of colchicine was used. The easy preparation of 10 % solutions would indicate that a chloroform-free substance was used. We have found, however, that the chloroform-containing substance may also be rather easily dissolved to 10 %, if the fluid is shaken or heated. The chloroform will then evaporate in part. Even in the clear solution undoubtedly enough of the chloroform may remain to give c-mitosis.

In order to elucidate this problem a little the present work was started. We tested *Colchicum autumnale* for its reaction to colchicine puriss. amorph. SANDOZ (without chloroform), to pure chloroform, to colchicine puriss. cryst. DAB 6 MERCK (with 12,5 % chloroform) and to

acenaphthene. Besides *Colchicum* we tested in a similar way the related species *Bulbocodium vernum*, belonging to the same section. Both experimental plants were bought from Weibullsholm, Landskrona.

The same simple method was employed in this work as in our earlier experiments. Bulbs with fresh root-tips in active growth were put into series of concentrations of the various substances. Only in a few cases (the testing of *Bulbocodium* with chloroform and some of the testings of *Colchicum* with chloroform) have we used another mode of procedure, this being made necessary by lack of plant material. Detached root-tips were submersed for 4 hours in solutions of the substances. Tests in *Allium* have shown that free roots thus treated during 6 hours give results fairly concordant with those obtained for attached roots.

The chromosomes of *Colchicum autumnale* have been studied by LEVAN (1940). The chromosome number is  $2n = 38$ . Great variations in chromosome size occur within the idiogram. Fig. 1 *a* shows a normal equatorial plate from an acenaphthene treatment. The chromosomes are somewhat contracted, which facilitates the counting. *Bulbocodium vernum* has been earlier studied by MILLER (1930), who gives its chromosome number as  $2n = 22$ . One plate from a 4 hours' treatment with 0.4 % colchicine cryst. is pictured in Fig. 1 *b*. Also here the chromosome contraction is somewhat stronger than normally. The plate is in this respect quite comparable to Fig. 1 *a*. The chromosomes of *Bulbocodium* are somewhat larger than those of *Colchicum*. Most of them are medially to submedially attached, but at least one of the smallest pairs is asymmetric with one arm and one small head. The size differences are less extreme than in *Colchicum*. The largest chromosomes of *Bulbocodium* are about  $5 \mu$  and of about double the length of the smallest ones. Although the idiograms of *Colchicum* and *Bulbocodium* are of the same general type, it is quite clear that no simple relation of polyploidy can exist between the two types. The largest chromosomes of *Colchicum* and *Bulbocodium* agree pretty well morphologically, but the smallest chromosome types of *Colchicum* are not present in *Bulbocodium*.

## II. EXPERIMENTS.

a. *Amorphous colchicine*. — This form is, compared with crystalline colchicine, highly soluble in water. Strong concentrations are, however, not stable, since after even a short time they form crystals of water-containing colchicine. This latter substance is soluble in water only to about 1.4 %. The strongest concentration of amorphous colchicine

tested by us is 20 % (0,5 mol; % = g substance in 100 cc solution). This solution kept clear for about 30 min., when it started crystallizing. On the first fixing (after 4 hours) the amount of crystals was estimated to be certainly below one-fourth of the dissolved quantity; so at that time the solution was still at least 15 %. Solutions down to 5 % were tested in *Colchicum* and down to 0,4 % in *Bulbocodium*. The toxic effect of pure colchicine is very insignificant in the two plants. A treatment of *Colchicum* for 11 days with an originally 20 % solution impeded, it is true, the further growth of the roots. Mitoses occurred, however, in the meristem, and they were quite normal. A fixation of *Bulbocodium* grown 4 days in the same concentration showed no perceptible toxic action. Although the root-tips were quite covered with colchicine crystals, they kept turgescient. In all lower concentrations mitoses were going on normally during the whole period of the experiment. No c-tumours were found.

We looked carefully for c-mitoses and signs of spindle destruction without any positive result. The mitoses and spindles were perfectly normal in all fixations. In the highest concentration a pronounced stickiness was met with, especially at the first fixing (after 4 hours); at the second fixing (after 24 hours) the stickiness was decidedly less marked. Fig. 1 c—f shows instances of mitoses from this treatment. As seen, the stickiness could be so severe as to cause lumping of the chromosomes, sometimes simulating certain c-mitotic features. The spindles were always present, however. In rare cases tendencies to small spindle disturbances were seen, as very wide poles with traces of multipolarity (Fig. 1 c—d). All anaphases studied were bipolar, however. Fig. 1 f shows all chromosomes gathered in the middle of a cell. It is strongly reminiscent of ball-metaphase but may possibly be an early metaphase stage, where the spindle has not yet acted on the chromosomes. This picture, which is very common in *Colchicum* roots showing c-mitosis, was very rare here. It must be concluded that no typical c-mitotic disturbances are induced by pure colchicine. The abnormalities seen in the highest concentrations may be ascribed to agglutination phenomena.

b. *Iso-colchicine*. — In *Colchicum* one experiment was made with *iso-colchicine* (concerning the effect on *Allium* of this substance, cf. STEINEGGER and LEVAN, 1947 a). Only one concentration was tested, viz. 1 % (0,025 mol). This rather strong solution (it is about half-saturated) gave no c-mitosis. It brought about a somewhat more pronounced stickiness than ordinary colchicine. The metaphases often

turned pycnotic and the anaphase chromosomes formed bridges (Fig. 1 g). The spindles were always normal. The substance had no great toxic effect, after 6 days the roots were still turgescant. They showed little further growth. No c-tumours were observed.

c. *Chloroform*. — Chloroform which accompanies colchicine in its crystalline form was tested in both plants in a series of the following concentrations: 0,5, 0,25, 0,10, 0,05, 0,025 % by weight. The activity threshold of *Allium* was known to lie at about 0,025 % (STEINEGGER and LEVAN, 1947 b). In *Allium* we found the strongest concentration to have a peculiar effect, it acted dissolving on the chromosomes, in extreme cases resulting in the complete disappearance of the chromosome arms, leaving the centromeres quite free. In *Colchicum* it turned out that one hour's treatment with 0,5 % chloroform gave the same effect. In *Bulbocodium* we fixed the material only once, viz. after 4 hours. Then the dissolution of the chromosomes had gone too far. The chromosomes at contraction stages had completely disappeared, the cells being quite empty. In the nuclei only the largest heterochromatic bodies were still to be seen. They formed 1 to 5 strongly stained bodies.

In *Colchicum* the various stages of the chromosome cycle were clearly seen. No c-mitotic changes occurred, this concentration of chloroform probably killing the cells before they were able to give any vital chromosome reactions. The chromosomes were at various stages of dissolution. Fig. 1 h—j shows one metaphase and two beginning anaphases. In h much of the chromatic substance is still gathered around the stained centromeres. In i the half-centromeres probably have not yet separated, in each chromosome they form two distinctly stained grains. They are smaller than the corresponding formations in *Allium* and they seem to be less compound morphologically, but, of course, with these small dimensions nothing can be said as to their detailed organization. In Fig. 1 j the centromeric points of each pair are sometimes rather far away from each other; they are evidently on their way to the poles. In this picture every trace of the chromosome arms had vanished and the centromeres themselves were faintly stained. It will seem that the dissolution proceeds in the same way as in *Allium*, taking away first the matrix substance, later the chromonema, and last the centromeres.

The next concentration, 0,25 %, is also lethal. The *Colchicum* roots were turgescant for a couple of hours in this concentration but after 4 hours they were already somewhat softened. This concentration also has a fixing action, but in *Colchicum* tendencies to c-mitoses were dis-

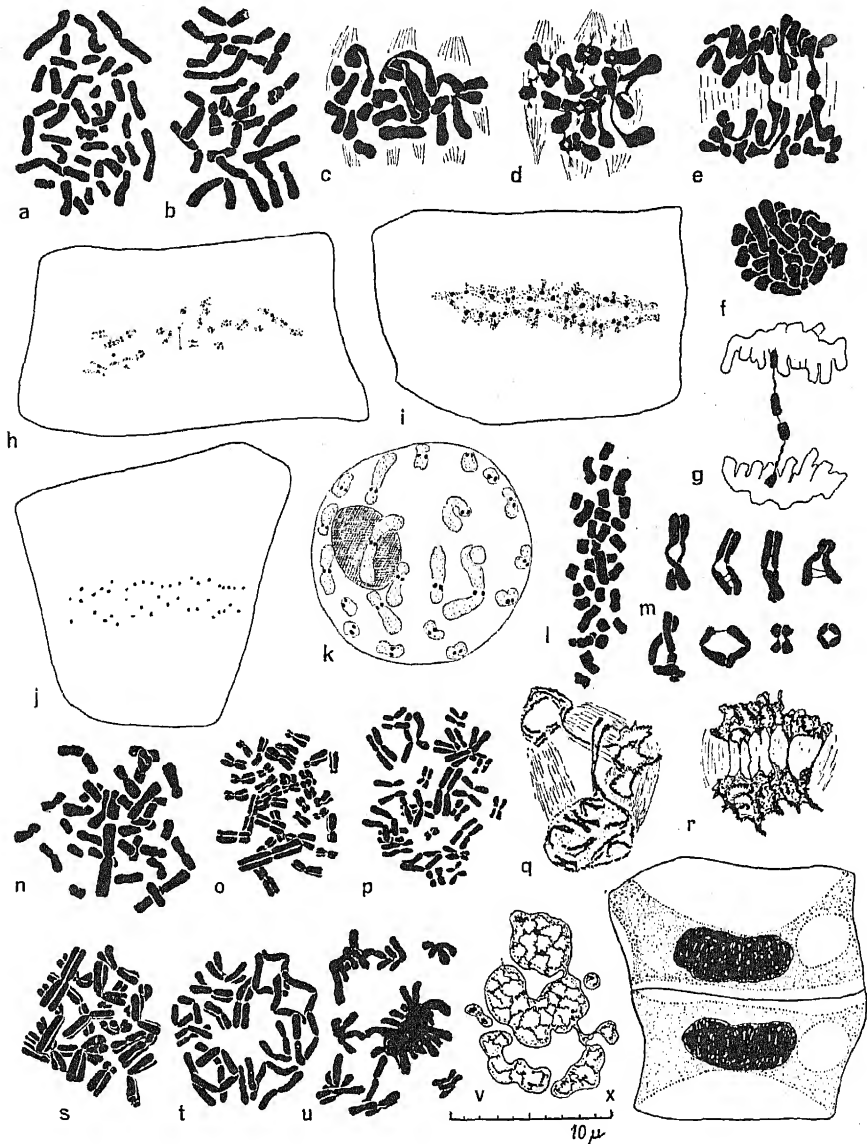


Fig. 1. a, c—r: *Colchicum autumnale*, b, s—x: *Bulbocodium vernum*; a—b: normal equatorial plates with chromosomes somewhat contracted from a slight c-mitotic treatment; c—f: treatment with amorphous colchicine; g: iso-colchicine; h—j: chloroform; l: crystalline colchicine; k, m—x: acenaphthene. —  $\times 2200$ .

cernible, since the chromosomes have had time to respond to the spindle destruction. Toxicity was very strong, all division stages being pycnotic.

In 0,1 % both *Colchicum* and *Bulbocodium* exhibited 100 % c-mitosis. Chromosome contraction was strong. Stickiness occurred but not to any high extent. The chromosomes were mostly lying free from each other, forming typical c-pairs. The concentration 0,05 % also gave full c-mitosis in *Colchicum* on testing detached roots. On testing roots still on the bulbs this concentration gave preponderantly normal mitoses. In *Bulbocodium*, where this series was made exclusively with detached roots, only few mitoses were present in this concentration, among them being both normal and c-mitoses. It is clear that both in *Colchicum* and *Bulbocodium* the activity threshold is approached: 0,025 % gives in *Colchicum* (detached roots) mixed c- and normal mitoses. *Bulbocodium* had no mitoses in this concentration. Thus, even if the lower limit of c-mitosis could not be exactly determined by these experiments, it is evident that it must be situated in the vicinity of 0,025 %, i. e. it is of the same size-order as in *Allium*.

Chloroform had a very pronounced toxic effect throughout all concentrations. In the lower concentrations it was especially clear after 24 hours, when no mitoses at all occurred. The highest concentration, 0,5 %, gave slack roots in less than 45 min. As mentioned already, the roots in 0,25 % began softening after 4 hours. After 16 hours they were quite soft. The 0,1 % solution also gave some defective turgescence after 5 days. None of the concentrations induced c-tumours, not even 0,05 %, which in *Allium* gave clear tumours.

d. *Crystalline colchicine*. — Solutions between 4 % and 0,5 % of chloroform-containing colchicine were tested in *Colchicum*, and between 4 and 0,01 % in *Bulbocodium*. These solutions are calculated to contain 12,5 % chloroform, which corresponds to chloroform concentrations of 0,5—0,06 % in *Colchicum* and 0,5—0,001 % in *Bulbocodium*. The toxicity of colchicine cryst. is much greater than of the same concentrations of colchicine amorph. It is somewhat less than that of corresponding concentrations of pure chloroform. The latter condition may be due to the calculated concentrations of chloroform actually being lower. Some chloroform may have evaporated at the preparation of the colchicine solutions. Moreover, the tests with colchicine were made with less fluid in lower vessels of larger surface in relation to the volume of fluid than when pure chloroform was tested, which may have brought about a relatively greater evaporation in the colchicine series. Thus, the smell of chloroform, which was very evident at first, had usually disappeared

totally after one day. The corresponding solutions of pure chloroform still smelt of chloroform after this time. The possibility that in some way or other the colchicine may abate the toxic action of chloroform cannot yet be rejected, however.

In the strongest concentration, 4 %, there should be present 0,5 % chloroform. This solution, however, has quite other effects than a pure chloroform solution of 0,5 %. It has not a fixing action. The roots of *Colchicum* were still turgescient on the third day, in *Bulbocodium* they softened on the second day. Chromosomal stickiness occurred, it is true, but not by far such an extreme dissolution of the chromosomes as in 0,5 % chloroform. In both species 100 % c-mitosis was found after 4 hours and the chromosome contraction was strong. Fig. 1 l shows a c-metaphase from this treatment. As early as after 24 hours, however, most mitoses had become normal, c-mitosis occurring only exceptionally. In *Bulbocodium* no divisions were found after 24 hours, the toxic action was such that divisions were very seldom found on the second occasion of fixation.

Solutions of 2 % and 1 % crystalline colchicine also gave 100 % c-mitosis in both species. In the latter concentration, though, other types of c-mitoses began to appear, viz. »star» and »distributive» metaphases, which indicates that the limit value is not far away. After 24 hours *Colchicum* showed preponderantly normal mitoses in 2 % and almost exclusively normal mitoses in 1 %. As mentioned above, *Bulbocodium* had no mitoses on the second day. The 0,5 % concentration had exclusively normal mitoses in *Colchicum*, as had 0,4 % and all lower ones in *Bulbocodium*.

C-tumours were demonstrated in *Colchicum* only in one concentration, viz. 0,5 % after four days. This agrees with the threshold of chloroform in *Allium*, where 0,05 % and 0,025 %, but not 0,1 %, induced c-tumours. A 0,5 % crystalline colchicine concentration contains 0,06 % chloroform, or probably somewhat less.

e. *Acenaphthene*. — This substance has earlier been tried on *Colchicum* only in qualitative tests. LEVAN (1940) dusted the roots with acenaphthene and let them thereafter grow in moist air. Under these conditions *Colchicum* was very prone to acquire typical c-tumours and c-mitoses. CORNMANN (1942) reports complete resistance to acenaphthene: »Mitosis continued normal in *C. autumnale* during 7 hours exposure to saturated aqueous acenaphthene» (l. c., p. 55).

The solubility of acenaphthene in water is about  $20 \times 10^{-6}$  mol/l (LEVAN and ÖSTERGREN, 1943). In a similar way as described in that

paper, solutions of acenaphthene were prepared, containing a varying excess of undissolved substance. The strongest concentration corresponded to a molarity of  $100 \times 10^{-6}$ . In *Colchicum* concentrations down to  $25 \times 10^{-6}$  mol/l were tested, in *Bulbocodium* we went down to  $1 \times 10^{-6}$  mol/l. In no concentration did 100 % c-mitoses occur. In *Colchicum* an exposure to  $100 \times 10^{-6}$  mol/l gave in 4 hours numerous complete c-mitoses with strong chromosome contraction. Various instances of c-mitoses from this treatment are pictured in Fig. 1 *n—r*. An often occurring stickiness is apparent both in the contraction stages of the chromosomes and, above all, in the bridge formation at anaphase—telophase (Fig. 1 *q—r*). There was a similar response in *Bulbocodium* to the same treatment. C-mitoses which end with multipolar anaphases and lobated telophase nuclei are common (Fig. 1 *s—v*).

*Colchicum* has a great frequency of c-mitoses in  $50 \times 10^{-6}$  mol/l as well. Stickiness is still evident, for instance, at the separation of the c-pairs at anaphase (Fig. 1 *m*). The centromeric region separates readily but the chromosome arms are glued together, strings of sticky chromatin are often formed between the chromosome arms on their falling apart. In *Bulbocodium* this concentration has a low frequency of c-mitoses, only single tendencies being seen. The  $25 \times 10^{-6}$  mol/l concentration in four hours shows in *Colchicum* still a few certain c-mitoses, in *Bulbocodium*, on the other hand, exclusively normal mitoses are found. The latter seems to be somewhat less sensitive to the c-mitosis reaction of acenaphthene than the former. Unfortunately no lower concentrations of *Colchicum* were studied, but it may be stated with great probability that the threshold of *Colchicum* is situated immediately below saturated solution of acenaphthene, while in *Bulbocodium* a certain excess of undissolved substance is necessary to attain the reaction.

One very interesting condition is characteristic of both *Colchicum* and *Bulbocodium*: the toxicity of acenaphthene, which after 4 hours is very small, is already quite pronounced after 24 hours. This is the case not only with the higher concentrations but goes down to the lowest ones tested (in *Bulbocodium* to  $1 \times 10^{-6}$  mol/l). In the entire *Bulbocodium* series hardly any normal division stages could be found on the second occasion of fixing. This was already seen macroscopically in that after a few days the roots grew brownish and ceased elongating even in the weakest solutions. As mentioned already, conditions were similar in the chloroform concentrations and to a certain degree in the colchicine cryst. series. In acenaphthene the roots kept turgescient and it happened that later on, after 4 days, new divisions began to appear,

although never in any great frequency. After 3 days clear c-tumours were started in *Colchicum* in 100 and  $50 \times 10^{-6}$  mol/l and tendencies to c-tumours in  $25 \times 10^{-6}$  mol/l. In *Bulbocodium* only very weak c-tumours were induced in 100 and  $50 \times 10^{-6}$  mol/l. It is evident, however, that cellular growth is not inhibited by the type of toxicity acting here.

Even in very low concentrations acenaphthene exerts a specific kind of toxicity on the meristems, a toxicity which is not known from the acenaphthene treatments of *Allium*. It takes an interesting expres-

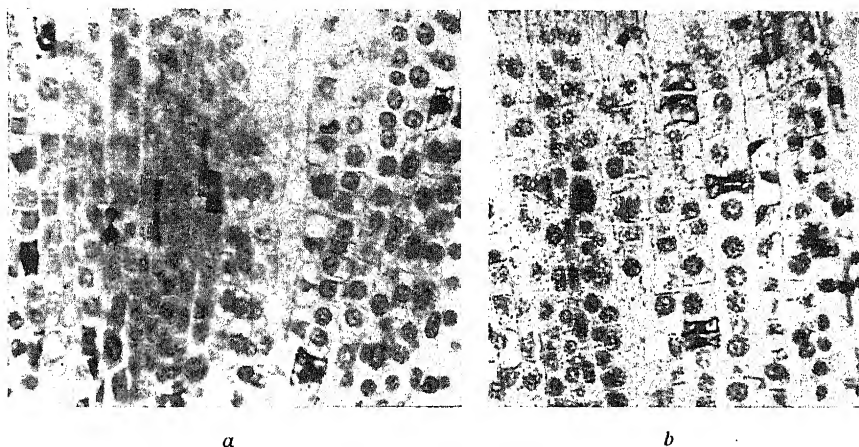


Fig. 2. Microphotographs of root meristems of *a: Colchicum*, *b: Bulbocodium* after treatment for 24 hours with acenaphthene. Single cells or pairs of cells are dead. —  $\times 250$ .

sion in the histology of the meristems. Certain cells die in the tissue. These cells are always rather isolated and, at least to begin with, the neighbouring cells are not affected. The meristem becomes scattered with solitary or paired dead cells. On a closer study of these cells it is found that they all have the common trait that they were in division stages of mitosis when dying, in metaphase or anaphase. Here evidently a selective cell lethality is in action, choosing cells in division. The threshold of this lethality lies far below the c-mitosis threshold. This toxicity is strongly specialized, distinguished from the general toxicity which impedes cellular growth. One instance of a couple of anaphase or telophase cells killed by this toxicity is pictured in Fig. 1 *w*. A tissue section of *Bulbocodium* and *Colchicum* affected by this type of toxicity is photographed in Fig. 2.

In the strongest concentration,  $100 \times 10^{-6}$  mol/l, there occurred in *Colchicum* after 4 days a very distinct centromeric staining during prophase. Fig. 1 k shows the lower half of one such nucleus with the chromosomes scattered on the nuclear membrane. All had their centromeres showing a distinct colour, while the rest of the chromosomes was faintly stained. As seen, the centromeres were double in transversal direction. They had about the same size and appearance as the »free» centromeres occurring after 0,5 % chloroform treatment.

### III. DISCUSSION.

The present experiments have given conclusive evidence that both *Colchicum* and *Bulbocodium* are resistant to colchicine. Their resistance keeps in concentrations as high as 20 %. The mitotic disturbances occurring in the highest concentrations of amorphous colchicine are not c-mitotic, but they are caused by the agglutination phenomena, which are often called stickiness. These may be induced by a great many chemical substances, among them many c-mitotic substances. *Colchicum* is also found to be resistant to *iso*-colchicine, even in concentrations which in *Allium* give c-mitosis. *Bulbocodium* was not tested with this substance.

The two plant species are also resistant to chloroform-containing colchicine up to concentrations in which the chloroform content reaches above the threshold value of c-mitosis. Then complete c-mitosis is obtained with crystalline colchicine. The results of CORNMAN (l. c.) may be interpreted as depending on his using a chloroform-containing substance. With the guidance of our experiments it is even possible to calculate approximately the content of chloroform which the substance used by CORNMAN must have held: 5 and 10 % in some cases gave complete c-mitosis, 2,5 % only gave partial. Consequently, at the treatments the colchicine substance should have contained 1 to 2 % chloroform.

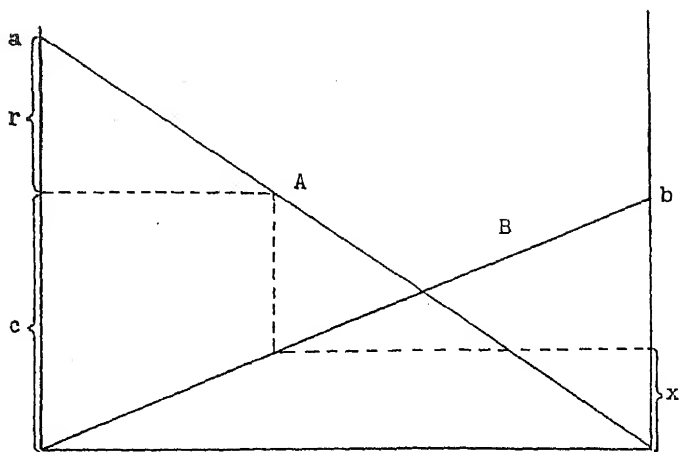
Even the strongest solutions tested of pure colchicine hardly act poisonously, while chloroform far down in the dilutions brings about considerable poison effects. Crystalline colchicine also acts toxically, although to a less degree than corresponding concentrations of pure chloroform. In *Bulbocodium* 4 and 2 % crystalline colchicine acted very poisonously, and a toxicity consisting in the inhibiting of all mitoses acted in colchicine concentrations of 0,01 %, i. e. a chloroform concentration of 0,001 %. What was especially surprising was the pronounced

toxicity of acenaphthene, which when used on other materials has shown very little toxicity.

The entire problem of resistance gains considerably in clearness if the c-mitotically active substances are classified into two groups, such with mainly unspecific, physical activity and such which in addition have a chemical activity, varying from substance to substance. While the substances of the former group probably induce the c-mitosis by some similar quality common to them all, the specifically acting substances of the latter group may attain their effect by attacks on any one of a number of different life processes of the cell; it may be a question of either a direct chemical influence on the components of the spindle or such an influence on ferment systems directly or indirectly involved in their synthesis. Only in their visible final effect do they agree in all cases: the morphological deviation of mitosis, called c-mitosis.

In a similar way it will be appropriate to divide the phenomena of resistance into two groups, unspecific, physical on one hand and specific, chemical on the other. A physical resistance (=decreased susceptibility), which, of course, can only be relative, may be due, e. g., to some deviating physical properties of the spindle constituents. The physical resistance may be extended to the whole group of unspecific («narcotic») poisons. This situation may be illustrated by the simple Scheme 1, constructed in accordance with ÖSTERGREN's schemes (1944, Fig. 11, p. 458). According to the hypothesis of ÖSTERGREN, the c-mitotic action is caused by an influence on the lipophilic side-chains of the polypeptid molecules. At a certain degree of «saturation» the chains are folded and the spindle formation is made impossible. ÖSTERGREN compares this process with the precipitation of a dissolved substance by adding another substance to the system. *a* and *b* of Scheme 1 represent molar concentrations of a saturated solution of the substances *a* and *b*. If the concentration of *a* sinks along the line *A*, it will be necessary, in order to obtain saturation of the solution, to add such a molar concentration of substance *b* as is expressed by the line *B*.

If this is adapted to the conditions of the c-mitosis, *a* will mean the «concentration» of lipophilic side-chains and *b* the concentration of a c-mitotic substance. Now, if *c* means the degree of «saturation» of the protein chains as regards lipophilic side-chains, the quantity *x* of the substance *b* necessary for inducing c-mitosis may be obtained by following the dotted line. From this it appears that the values of *x* increase simultaneously as *c* decreases. In other words: the farther the concentration of lipophilic side-chains is from its «saturation» (expressed by



Scheme 1. (See text.)

the distance  $r$ ), the more substance  $b$  must be added in order to obtain c-mitosis, the greater is consequently the resistance of the cell. This suggestion, naturally, is not necessarily linked to the hypothesis of »protein chain folding», but it will work equally well on any hypothesis assuming such a precipitation of lipophilic groups or molecules.

This situation is, of course, valid only with physically acting substances. The chemical resistance, on the other hand, must vary according to the point of attack of each particular substance. In each single case a certain resistance must be mobilized, directed to the specific attack in question. Thus, immunity to one chemically acting substance does not mean immunity to all substances of this group.

The following table may serve as an illustration of these conditions. As regards the chemical activity of  $\text{CHCl}_3$ , see our earlier paper (1947 b).

Group	Substance	Threshold concentration in %		
		<i>Allium</i>	<i>Colchicum</i>	<i>Bulbocodium</i>
more or less chemically acting	colchicine ...	0,004	$> 20$	$> 20$
	chloroform...	0,025	0,025	0,025
preponderantly physically acting	iso-colchicine	0,6	$> 1$	?
	acenaphthene	0,000.08	0,000.4	0,000.8

As representatives of specifically acting substances colchicine and chloroform are given. The difference in behaviour between *Allium*, on

one hand, and *Colchicum* and *Bulbocodium*, on the other, is striking. While the latter species are completely resistant to the chemical influence of colchicine, they are as sensitive as *Allium* to the influence of chloroform. Their chemical resistance is accordingly specific. Further, two instances of preponderantly physically acting substances are given: *iso*-colchicine and acenaphthene. *Colchicum* is somewhat more resistant than *Allium* to acenaphthene, and the same is the case with *iso*-colchicine, which substance has, it is true, quite another chemical nature than acenaphthene but nevertheless acts preponderantly unspecifically. Although the values so far at hand are insufficient to permit any proof of the present view of the resistance problem, they must be considered as rather suggestive. LEVAN and ÖSTERGREN's results in *Allium* and *Pisum* (1943) may also be mentioned:

	Threshold value in $10^{-6}$ mol/l		
	Colchicine	Naphthalene	Acenaphthene
<i>Allium</i> . . . . .	150	78	5
<i>Pisum</i> . . . . .	75	156	32

These values also are not inconsistent with our view that chemical and physical resistance are independent of each other. While *Pisum* is more sensitive to chemical influence than *Allium*, its resistance to naphthalene and acenaphthene is greater. It seems as if an increased resistance to acenaphthene may also involve a similar increase in resistance to naphthalene. The unspecificity of the resistance to physical action is also present in the «camphor reaction» of yeast (LEVAN, 1947). An induced resistance to certain substances also brings about an increased resistance to other chemically quite unrelated substances.

### SUMMARY.

The reaction of *Colchicum autumnale* and *Bulbocodium vernum* to amorphous (pure) and crystalline (chloroform-containing) colchicine, *iso*-colchicine, chloroform and acenaphthene is studied. Both species show an absolute resistance to the c-mitotic action of colchicine and *iso*-colchicine in high concentrations (20 and 1 % respectively). To chloroform their response is similar to other colchicine sensitive plants as, for instance, *Allium Cepa*. They both give typical c-mitoses if treated with such concentrations of crystalline colchicine as have a chloroform content above the threshold concentrations of this substance alone. Both

are susceptible to the c-mitotic action of acenaphthene. As explanation of the data on the susceptibility of *Colchicum* to colchicine encountered in the literature it is suggested that a substance containing chloroform may have been used. In agreement with the two types of action on the living cell of c-mitotic substances, viz. physical and chemical action, it is suggested that two types of resistance may occur: a physical, less selective resistance and a chemical specific resistance, which in each case is directed against a certain kind of attack. While in *Colchicum* and *Bulbocodium* the resistance to the chemical activity of colchicine is absolute, they are both as susceptible as *Allium* to another very probably chemically acting substance, chloroform. Both species are more resistant than *Allium* to acenaphthene and iso-colchicine (only *Colchicum* studied), which are mainly physically acting substances.

Svalöf, March, 1947.

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## ABSTRACTS — KURZE MITTEILUNGEN

GÖSTA VON ROSEN: The rapid nigrosine-method for chromosome counts, applicable to all the growing tissues of the plant.

A plant-breeder who works with cross-fertilizing kinds of plants and who desires to work up polyploid material on a large scale will soon discover that an annual control of the purity of the polyploid stocks as regards chromosome number is necessary. For even if he isolates the polyploid materials well by distance from other propagations, there will be so much pollen flying about in the air (e. g., in *Beta*) that some risk of incrossing with undesired chromosome numbers will constantly be present. A breeder of fruit and forest trees should also have at his command methods by which the chromosome numbers in new artificial or spontaneous crosses can be simply separated.

A grading of the polyploid chromosome-number groups only demands an estimation of the number of chromosomes in the genome (e. g., in *Beta*  $36 \pm 2-4$ ). If, in addition, the method can often give metaphase plates so well pressed out that an exact count is possible, this is obviously an advantage. First and foremost, however, it must provide a rapid means of getting the preparations ready and of reading them (= well contracted chromosome bodies). The usual paraffin-gentian method as well as the Feulgen-smear method (e. g., JOHNSON, 1945; MEYER, 1943; WHITAKER, 1939) are too time-absorbing to be suitable for serial work. There are numerous variations of smear preparation with carmine or orcein as stain (e. g., BROWN, 1937; BURREL, 1939; VON ROSEN, 1946 a, b; SAX, 1931; SMITH, 1947; STEERE, 1931; THOMAS 1940; WARMKE, 1935; WHITAKER, 1934). All suffer, however, from the weakness that they only stain the chromatin red and in addition give a poor differentiation between chromatin and plasm. Heating is also included in the process of preparation, which makes the work delicate. Further, chromosome counting ought to admit of being done during the whole vital cycle of the plants, this enabling a failure at the beginning to be remedied later on. In *Beta*, for instance, now at Hilleshög the rootlets are forced artificially during the winter, and an after-control can be carried out later on leaves from the stem-tips from the commencement of growth up to the flowering. The pollen mother-cells can also be rapidly judged. *All this ought to admit of being done by the same simple method.* It would mean that dear, specially trained staff would not be required for the purpose, as a small number of persons engaged in other work during the rest of the year have good time at their disposal to carry out the program.

A rapid method for the plant-breeder for the grading of polyploid material should be of the following nature: (1) simple, (2) distinctly contrast-staining, (3) rapid, (4) adapted to serial work, (5) applicable to all the growing tissues of the plant, (6) cheap.

The method described below seems to meet these requirements. The treatment is essentially the same for roots as for leaves and flower-buds. Any deviations between them are noted in their proper connexion.

(1) *Fixing.* — Fix the material in 1 part of concentrated (98 %) acetic

acid + 2 parts of 95 % alcohol for about 24 hours. The preparation should be preserved in a cool state (not above 15° C., preferably 8—12° C.). The fixing solution must be mixed within 24 hours of being used.

(2) *Loosening of the tissues*. — Replace the fixing fluid with a mixture of 1 part of concentrated HCl (sp. gr. 1.27) + 2 parts of 95 % alcohol. Roots are treated for 8—10 minutes at a temperature of 10—15° C. (an important condition is that the fluid is cooled). For leaves the character of the tissues will decide the concentration and time: *Beta* 1 : 2 for 7—8 minutes, *Pisum* 1 : 4 for 5 minutes, *Pyrus* and *Betula* 1 : 1 for 10 minutes, and so on. Flower-buds are treated with 1 part of HCl + 10 parts of alcohol for 5—8 minutes (for *Beta* and some other plants). The HCl-alcohol mixture can only be used one day.

(3) *Washing*. — Replace the loosening fluid with distilled water (cooled) and preserve the preparation in a cool state (8—12° C.; not above 15° C.). Washing is completed within 15—30 minutes. For the preparation of thick leaf-tissues and body complexes 60 minutes should give a more reliable result.

(4) *Staining*. — Place 4—5 roots on an object-glass. In daylight or light from a daylight lamp against a black background the zones undergoing brisk division in the root-tip can be distinctly seen. Cut away portions not showing divisions. Suck off the water with filter-paper. Dry leaves and bodies by laying them out on a filter-paper, then place them on the object-glass. As a leaf-specimen take a *small* piece from the base of a leaf on a briskly growing part of the stem. Squeeze flower-buds into pieces, whereupon rather large portions of the bracts are picked off with a pincette. One drop of staining solution (for preparation, see below) is added. Put the tissues into the fluid and place a cover-glass loosely over. Lay the object-glass on a thick, smooth plate of glass. The subsequent procedure is as follows: with an ordinary piece of stiff eraser rubber (a) tap gently on the cover-glass to ensure the fluid making contact with the tissue, then (b) press fairly gently over the whole cover-glass for short periods so that the fluid is pressed outwards to the edges. When the white tissues that have now been squeezed a little out are clearly visible, press (c) hard with short vibratory movements directly above the root-tips, though not so that the cover-glass slides. Now (d) lift the cover-glass cautiously by one edge, though only so much that the staining solution flows in over the tissues. Considerably less pressure is used for leaves; the divisions in the parenchyma are to be disclosed. Pollen mother-cells are pressed still more mildly. For roots the staining time is 1—2 minutes. The staining fluid should be cool, about 15° C. During the period of staining the preparation tolerates normal room temperature (16—18° C.), but must not be heated with a strong lamp or similar source of heat. Leaves of *Pisum* are stained for 30 seconds, those of *Pyrus* and *Betula* for 45—60 seconds. For these types of tissue 4 % staining fluid is used. Buds, on the other hand, require staining for 4—5 minutes in a 3 % solution. When staining pollen mother-cells care should be taken not to lift the cover-glass as the large pollen mother-cells may thereby be damaged. The pigment colours the chromosomes quite black and the plasma a faint grey.

When the preparation has been stained, lay a filter-paper over the preparation glass and pass a rubber-roller to and from some ten times over

the cover-glass, preferably also crosswise. Over the root-tips roll at first gently, then rather hard; on leaves rather gently the whole time and on the pollen mother-cells very gently. A good preparation should not be vesicular and the tissue ought to be squeezed out to at least fivefold its size.

The preparation is then ready for immediate microscopical determination. In cool temperature it will keep for 24—36 hours, leaf preparations for 2—3 days. For work in green light it is suggested that a white dull-glass filter is to be placed before the lamp and a thin green glass in the iris diaphragm of the microscope. The chromosomes now will show up quite black and the plasm colourless, but some of the luminous brightness is thereby lost.

(5) *Staining fluid*. — Prepare a 4 % solution as follows: dilute 50 c. cm. of concentrated (98 %) acetic acid with distilled water to 100 c. cm., so that the fluid contains about 50 % of acetic acid. Heat this solution to boiling-point. Add, continuously stirring the while, 4 gms. of *spirit-soluble nigrosine* (see Stain-Technology, vol. 10, p. 73, 1935). The black staining fluid is to boil for 3—5 minutes, i. e. so long that it acquires a weak farry consistence. When large batches are prepared, careful allowance should be made for the evaporative affect of the larger boiling volume, the boiling intensity, and so on. On removing the solution from the source of heat allow it to cool a few minutes at room temperature. Then cool it rapidly to room temperature and filter it immediately, taking care to avoid unnecessary evaporation. Keep the solution in a closed glass vessel in the dark under room temperature for about 10—14 days before use.

The above-described method is now being used at Hilleshög in actual practice. A girl gets the preparation ready, another one carries out the microscopical work; both were previously quite unacquainted with cytological and similar technique. After two or three days training their capacity was 60 plant-counts on root-tips per working day of seven hours. To-day this number is increased to 100—120. Leaf-counts are performed quicker than root-counts (150—200 slides per day).

Finally, it may be mentioned that the writer has conducted tests in *Abies* and *Pinus*. Counts were made without difficulty even in these genera, which are considered to offer difficulties for cytological work.

Beet Breeding Institute of the Swedish Sugar Company at Hilleshög, Landskrona. March, 1947.

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ARNE LUNDQVIST: On self-sterility and inbreeding effect in tetraploid rye.

The present investigation was started in the spring of 1945. Isolations (parchment baggings) were made both on diploid and tetraploid »Stålråg», a well-known commercial variety, from which a tetraploid strain was raised by Professor A. MÜNTZING more than five years ago.

As regards the self-sterility, this has turned out to be uneffaced in this material of tetraploid rye, but a weakening of the incompatibility is noticed, especially if the category with the lowest self-fertility (0—25 %) is taken into account.

TABLE 1. *Percentage of seed setting after selfing.*  
( $F_1$  = isolations giving rise to a  $I_1$  generation.)

		0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	100	n	$\bar{X}$
	a $F_1$ 1945	....	47																	47	2,50
2n	b $F_1$ 1946	....	140	5	1	1		1		1	3		5	6	4	5	7	3		182	13,98
	c $F_1$ 1942	....	160	8	1	1	1			1			1							173	3,48
4n	d $F_1$ 1945	....	65	11	3	2														81	3,92
	e $F_1$ 1946	....	158	12	8	4	3	1	1											187	4,18
2n	f $I_1$ 1946	....	52	1	2							1	1	3	1					61	8,16
4n	g $I_1$ 1946	....	94	14	3															111	3,40

The high average degree of self-fertility in the *b* series is caused by the great number of individuals with percentages ranging from 50 to 80. Their occurrence is probably due to the fact that the material of kernels from which the *b* series was raised was obtained from plants in standard plots in a large material of »Stålråg» that had inbred for about 20 generations and had reached a high degree of self-fertility. Thus, the progeny of this standard material must have been infested with factors promoting self-fertility. For the sake of comparison data reported by MÜNTZING (1943) are included in the *c* series.

There is a close agreement in the lower classes of fertility between the

$\alpha$ ,  $b$  and  $c$  series. The  $\alpha$  and  $d$  series were compared; a  $\chi^2$  test applied to the classes 0—5—100 gave a  $\chi^2$  of 10,624 and a  $P$  slightly larger than 0,001. The same comparison between the  $c$  and  $e$  series applied to the classes 0—5—10—100 gave a  $\chi^2$  of 6,840 and a  $P$  intermediate between 0,05 and 0,02. This difference in self-fertility is the more significant as the fertility after open pollination is lower in the tetraploids (about 80 % in the diploids, 60 % in the tetraploids).

The data of the  $I_1$  series  $f$  and  $g$  also indicate a weakening of the incompatibility in the tetraploids. The occurrence of individuals in classes of high fertility in the diploid series, however, results in an average more than twice the value of that of the tetraploids.

Regarding the influence of inbreeding, both the diploids and tetraploids show inbreeding degeneration, but the effect, though quite considerable, is almost in each case less marked in the tetraploids. Regarded separately, the differences are frequently not highly significant, but the significance is to be found in the agreement of the results. Lack of space does not permit a report of all results from comparisons in various characters, but in order to demonstrate the general tendency the data from the two characters showing the greatest difference between diploids and tetraploids in inbreeding effect after one generation of selfing are given below.

TABLE 2. *Plant height in cm. Diploids and tetraploids.*

	40	50	60	70	80	90	100	110	120	130	140	150	160	n	$\bar{X}$	m
2n Pop. 1946 ....					1			12	21	37	52	27	4	154	129,42	1,05
2n $I_1$ 1946 .....			3	3	7	12	15	13	8	4	2			67	105,30	2,27
4n Pop. 1946 ....				1	1	5	13	17	56	47	13	2		155	125,71	1,09
4n $I_1$ 1946 .....	1	1	1	1	6	3	16	22	30	11	1			93	114,25	1,84

TABLE 3. *Average number of spikelets per ear. Diploids and tetraploids.*

	0	5	10	15	20	25	30	35	40	45	n	$\bar{X}$	m
2n Pop. 1946 .....					1	8	20	71	49	5	154	33,15	0,37
2n $I_1$ 1946 .....			2	1	3	8	16	31	5	1	67	28,92	0,81
4n Pop. 1946 .....				2	4	8	31	77	31	2	155	31,47	0,41
4n $I_1$ 1946 .....			2	3	2	8	21	47	10		93	29,54	0,65

I hope soon to be able to treat these questions more in detail and to present all the results. As a rather limited first  $I_1$  generation has been the base of this investigation of the effect of inbreeding (25 lines in the diploids, 32 in the tetraploids), the experiments will be repeated on a larger scale and extended in various ways.

Institute of Genetics, Lund, Sweden, February 22nd, 1947.

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NILS NYBOM: Accessory chromosomes in *Allium*.

In a small sample of *Allium Porrum* plants the present writer found an inflorescence the pollen grains of which contained a varying number of ac-

cessory chromosome elements, at least of two different sizes. Fig. 1 shows a pollen grain with one long (not yet identified) and five short accessory chromosomes in addition to the 16 normal ones.

Preliminary studies show that these accessory chromosomes undergo a normal disjunctional division at the pollen mitosis. Thus, they have not the ability of the B-chromosomes of *Secale* and *Anthoxanthum* to increase their number by preferential non-disjunction at the pollen mitosis.

Accessory chromosomes are known from a great number of plants and animals. However, the question of their properties and function has acquired a new significance through the contributions of MÜNTZING (e.g. 1946) and

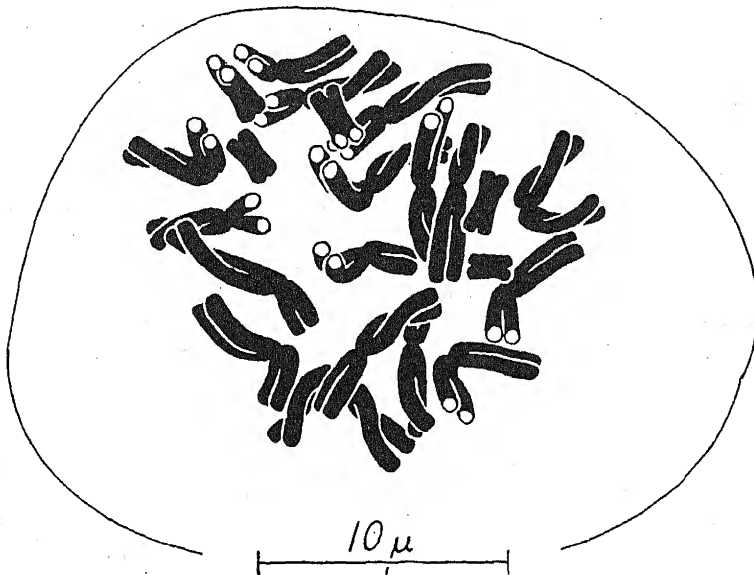


Fig. 1. A pollen grain with accessory chromosomes.

ÖSTERGREN (1945, 1947). As a part of the rather extensive works on B-chromosomes that are carried out and designed at the Institute of Genetics, Lund, the present writer intends to perform a cytological investigation of the seed material originating from the *Allium* plants mentioned.

Lund, Institute of Genetics, February, 1947.

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3. — 1947. Heterochromatic B-chromosomes in *Anthoxanthum*. — *Hereditas* XXXIII: 261—296.

TYGE W. BÖCHER: Cytological studies of *Arabis Holboellii* HORNEM.

In a list of chromosome numbers of arctic and boreal plants (BÖCHER, 1938) the haploid number  $n = 22$  for *Arabis Holboellii* was mentioned. This count was based upon investigations of a large number of metaphase plates of the first pollen mitosis. Later, a number of counts were made by ROLLINS (1941) in pollen mother-cells of *Arabis Holboellii* var. *retrofracta* and var. *pinetorum*. According to ROLLINS, var. *retrofracta* has  $n = 7$  and 14, and var. *pinetorum*  $n = 14$  and 21. As pointed out by ROLLINS, the number  $n = 22$  is not compatible with the numbers found by him, and this fact makes more cytogenetic work of this species necessary.

In order to clear up some of the problems I have collected and studied more material of the species. This material, as well as that studied earlier, was from the Botanical Gardens in Copenhagen. The plants cultivated here are undoubtedly descendants of plants from West Greenland. They belong to *Arabis Holboellii* var. *typica*, which occurs in Greenland and in northern North America.

In the first pollen mitosis 22 chromosomes, or perhaps more correctly  $21 +$  a very small extra chromosome, were counted again. But the same number could further be established in root-tip mitosis, in some few embryo-sac cells, and in some cells in embryos. In the metaphases in the PMC's a varying number of bivalents, trivalents, and univalents are present. In several cases, however, complete asynapsis occurred. In most pollen-sacs both meiotic divisions were omitted. The PMC's form a kind of restitution nucleus with  $21 + 1$  chromosomes. Thus, the pollen-grains with  $21 + 1$  chromosomes are monade-pollen and originate from unreduced nuclei containing the somatic chromosome number. In some of the pollen-sacs studied, however, dyads are also produced, resulting in smaller pollen-grains which frequently degenerate.

The material strongly supports the idea that *Arabis Holboellii* var. *typica* is a triploid type containing an extra chromosome of small size. Furthermore, apomixis is very probable. Some castration experiments show that pollination is necessary for seed production. Hence the plant may be pseudogamic.

A more detailed paper on the subject is planned and may appear when studies on some new material collected in 1946 in different localities in West Greenland have been completed.

Plant-Anatomical Institute, University of Copenhagen, March, 1947.

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ÅKE GUSTAFSSON: The advantageous effect of deleterious mutations.

Lethal mutations are common in cross-fertilizing populations of plants and animals. Owing to their recessive character they do not immediately show

up in nature, but they readily become unmasked in laboratory experiments. Often such mutations consist of short deficiencies, inactivating or destroying part of the chromosome substance. They are generally regarded as loss mutations, draw-backs in evolution, having no positive value whatever.

In 1946 the author argued that this latter conclusion did not fit the facts. On the contrary, many lethals increase the individual viability and plasticity in heterozygous condition, although they destroy the organism in homozygous state. Lethal genes are not merely negative; some of them act as valuable tools in the dynamics of the species.

Exact model experiments are difficult to carry through in cross-fertilizing organisms, even in *Drosophila*. They require homozygosity of most or all genes, except the deleterious one. Viability and competitiveness must be precisely and adequately measured. Fertilization by controlled gametes ought to take place. An agricultural plant like barley immediately fulfils these requirements. In the pure line Golden barley, isolated in 1897, several spontaneous chlorophyll mutations have been obtained. Two of them, *albina* 7 and *xantha* 3, have been repeatedly used in experiments (GUSTAFSSON, 1946). The monohybrids of these lethals show a distinct superiority to their normal sister plants, although the differences require a large material in order to be definitely proved. An obvious feature is, however, their longer preservation of seed germination.

Some years ago these two mutations were combined to form a dihybrid. They segregate independently of each other. No hybrid sterility is present. All plants of a large dihybrid offspring were analysed in 1946—1947 with regard to the number of spikes per plant, number of kernels per plant and kernel weight per plant. Afterwards the genotype was determined for every plant in the offspring. The results are shown in Table 1.

Previous analyses of monohybrid offsprings indicated a perspicuous though small heterosis effect. This becomes strikingly increased in the dihybrid condition. The two deleterious mutations add their effects. Spike

TABLE 1. *The increased viability of chlorophyll heterozygotes in a dihybrid offspring.*

	No. of spikes per plant	Prop.	No. of kernels per plant	Prop.	Kernel weight per plant (gram)	Prop.
Dihybrid ( <i>AaBb</i> ) .....	$5.47 \pm 0.17$	1.19	$114.16 \pm 3.82$	1.18	$4.54 \pm 0.16$	1.15
A7-monohy- brid ( <i>AaBB</i> )	5.05	1.10	104.76	1.08	4.17	1.06
X3-monohy- brid ( <i>AABb</i> )	4.88	1.07	104.66	1.08	4.20	1.06
Normal type ( <i>AABB</i> ) .....	$4.58 \pm 0.22$	1	$96.62 \pm 4.01$	1	$3.95 \pm 0.23$	1
Diff. dihy- brid/normal type .....	$P = 0.001$		$0.01 > P > 0.001$		$0.05 > P > 0.02$	

number is heightened by 19 %, kernel number by 18 % and kernel weight — the property of special agricultural value — by 15 %. The differences between dihybrid and normal type (Golden barley) are statistically significant. The increase in total kernel weight becomes even more remarkable, taking into account that 38 % of the seeds of the dihybrid plants are recessive for one of the mutations and 6 % for both of them.

JONES (1945) published important data on monohybrid heterosis in inbred lines of maize. These lines have no immediate value as yield varieties. Golden barley, on the other hand, was for several years the best two-row barley in North Europe and is still locally cultivated. All the best Scandinavian cross varieties (Kenia, Maja, Balder, Ymer) are partially built up on its genotype. After thirty years of continuous work the barley yield has been increased by about thirteen or fourteen per cent above the level of Golden barley. What such prominent plant-breeders as HANS TEDIN, VESTERGAARD, NILSSON-EHLE did not fully accomplish — the fifteen per cent increase —, is here in principle achieved by two deleterious mutations.

A further analysis shows that dihybrids and monohybrids are specially apt to profit by favourable environmental conditions. More readily than their normal sister plants they produce a high spike and kernel number, as well as a high kernel weight. They increase the range of variation, augment the plasticity (Table 2). Also here the monohybrids lie midway between the dihybrid and the normal state.

TABLE 2. *The increased variation in positive direction of dihybrid and monohybrid chlorophyll mutations.*

	No of spikes per plant			No. of kernels per plant			Kernel weight per plant (gram)			No. of plants
	> 7 %	> 8 %	> 9 %	> 180 %	> 210 %	> 240 %	> 7 %	> 9 %	> 11 %	
Dihybrid .....	17,6	13,3	8,2	12,9	6,9	3,9	13,3	5,6	1,3	233
Monohybrids (average) ...	11,3	6,0	3,5	7,0	2,5	2,1	10,2	3,2	0,4	284
Normal type	8,6	2,5	0,0	6,2	2,5	0,0	8,6	2,5	0,0	81

In consequence, a genotype with half its offspring having lethal character can be conspicuously superior to its normal relatives. And — the fundamental fact — this superiority is effected by the deleterious mutations themselves. This advantageous effect probably holds true of numerous lethals in natural populations of plants and animals, man not to be forgotten.

Svalöf, Institute of Genetics. April, 1947.

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